THE JOHNS HOPKINS UNIVERSITY

SCHOOL OF MEDICINE

FINAL STATUS REPORT

BIOLOGICAL EXPERIMENTS IN SPACE

PSYCHOCARDIOVASCULAR REACTIONS DURING CONDITIONS OF WEIGHTLESSNESS IN AN ORBITING SATELLITE

FROM OCTOBER 1963 TO MARCH 1968

Baltimore, Maryland 21205

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PSYCHOCARDIOVASCULAR REACTIONS DURING CONDITIONS OF WEIGHTLESSNESS IN AN ORBITING SATELLITE

From October 1963 to
March 1968

Dr. Jorge Perez-Cruet, B.S., M.D., Responsible Investigator
The Pavlovian Laboratory, The Johns Hopkins University
School of Medicine

Contract Number NsG-520

Supported by:

National Aeronautics and Space Administration

With technical assistance from:

Mr. Michel Zeltzman, Mr. Raymond Bannar, Mrs. Patricia Thiess, Miss Elizabeth Craig, Miss Maura Gage, Mrs. Lilliam Kagen, Mr. Richard Ferris and Mr. Andrew Calabrese.

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ACKNOWLEDGEMENTS

The principal investigator is very grateful for the consistent support from Ames Research Center, Moffett Field, California, and the Bio-Science group in NASA Headquarters, Washington, D.C. I am particularly thankful for the constant encouragement given to me by Drs. Dale W. Jenkins, Orr E. Reynolds, Joseph F. Saunders and Richard Belleville from the Bio-Science group and Drs. Thom Verhave, Robert Schiffman, G. Dale Smith and other professional personnel from Ames Research Center, Moffett Field, California. I am also grateful to several experimenters in the Bio-Satellite Project in particular Drs. Ross Adey, Nello Pace and John P. Meehan, who have been kind to listen to my work, influenced by scientific approach and given me a lasting example of patience in scientific endeavors of this kind.

INTRODUCTION

Our overall objective remains to increase our basic knowledge of the possible detrimental effects, if any, of prolonged Space travel and weightlessness on the nervous control of circulation (psychocardiovascular reactions). This investigation is important in the initial attempts to define and identify the problems of immediate or latent effects on the cardiovascular system produced by very prolonged Space travel conditions and prolonged periods of weightlessness, and for the further development of scientific methods for prevention of such cardiovascular effects in future prolonged Space flights.

Specifically: 1) cardiovascular measurements are being made in several parameters and simultaneously recorded. These are observed for several weeks in primates restrained in monkey chairs and in unanesthetized dogs. More recently, we are trying to allow some of the preparations to be in freely moving conditions. Various patterns of the cardiovascular responses have been analyzed for future correlation with changes due to prolonged weightlessness. Also analysis of changes in these parameters are described as they occur in relation to a given stimulus; 2) other measurements of renal and excretory functions have been recently incorporated and correlated with the cardiovascular measurements; 3) the studies have been extended to other species of animals in order to have a more economical approach to some of the problems in the development of techniques for chronic experiments; and 4) new developments in transducers for data analysis systems are currently being used in this laboratory as an ancillary part of the experiments.

Initially, most of the research effort was concentrated on the development of techniques for eventual use in Space conditions without technical handling of the specimens. Although some research effort is still being performed in this area, the main effort now is diverted to the development of accurate measurements of cardiovascular functions and investigation of the effects of external and internal stimuli on these functions. We consider this to be an important aspect of our overall objectives which will give valuable information concerning certain aspects of the nervous control of circulation under weightless conditions in an orbiting or interplanetary satellite.

CONCEPTUAL BACKGROUND OF THE EXPERIMENTS

The present experiments were designed in the early part of 1963 at which time plans for a Bio Satellite Project were started. At that time little was known about the possible effects of weightlessness on cardiovascular functions. Information available to us indicated that a number of non-specific cardiovascular reactions occurred during space flights. However, the main concern of the American and Russian programs was to develop quickly adequate space technology in propulsion and vehicular designs. Scientific biomedical reports from the Russian flights (Parin, et al., 1965) indicated that a number of cardiovascular changes occurred during orbital flights. The Russians thoroughly influenced by Pavlovian psychophysiology interpreted some of these functional changes in the cardiovascular system during weightlessness as being impossed by environmental and conditional changes on "higher nervous activity". The Pavlovian concepts of orienting (novel) and conditional (acquired) reflexes were implied in some of these interpretations. Parin and collaborators (1965) found that definite cardiovascular changes indicative of increased vagal tone occurred during weightlessness. They reported consistently slower heart rate during weightlessness and defined the cardiovascular changes during take-off and re-entry.

Animal experimentation by (Frank, et al., 1965, Chernov & Yakolev, 1959 & Henry, et al., 1952) indicated that during the take-off, re-entry and weightlessness there were stress changes in the cardiovascular system on non-specific origin. However, the Russians, in rocket flights, showed that some of the changes observed during take-off and short periods of weightlessness were dependent on the integrity of the nervous control of physiological functions. Our chimpaze experiments (Henry, et al., 1963) did not show significant changes in the various biological systems tested but the experiments were so complex in nature that no clear patterns could be detected, nevertheless the data did not show any clear-cut effects on cardiovascular functions except of the stresses produced by the conditions of the flight.

More recently there is a strong impression that there are no deleterious effects of prolonged weightlessness (14 days) on cardiovascular functions. However, this impression will have to be supported by more flights of this nature. The fact that there is no pathological effects of weightlessness on cardiovascular function do not preclude that the nervous control of circulation is not affected during flight. The fact that an astronaut in G-VII showed minor cardiac impairment during a tilt table test after 14 days of weightlessness could not be taken as a strong indication that weightlessness had any pathological effects on cardiovascular functions, but it suggests that there were some possible changes in the neural control mechanisms.

It is my opinion that the problem of neural control of circulation and the effects of psychic events on cardiovascular functions under conditions of prolonged weightlessness had not been studied thoroughly and that these scientific questions will have to be explored in the future. Two main questions remain unanswered. Does prolonged weightlessness during orbital flights modify the responsivity of the cardiovascular system to new psychic and environmental demands? Does prolonged weightlessness in interplanetary travel modify the responsivity of the cardiovascular system to new psychic and environmental

demands? Are these two "weightless" conditions different? How are we to refute these mechanisms when the experiments in the past flights have not answered these questions.

We have shown previously that the study of cardiovascular conditioning require rigid and exquisite controls under terrestrial conditions. It was Pavlov (1928) who showed that in order to study conditional reflexes the subjects have to be isolated in special sound proof chambers and the effects of the environment has to be rigidly controlled. (In this respect a vehicle in space will be an ideal laboratory). The field of cardiovascular conditioning and the study of psychophysiological reactions of the cardiovascular system is increasingly becoming more sophisticated and predictability of some psychocardiovascular functions, under certain limitations, is possible at present. This is supported by the proliferation of investiations in numerous laboratories concerning cardiovascular conditioning and psychophysiologic reactions of the cardiovascular system.

The study of cardiovascular functions accompanying orienting reflexes and classical cardiovascular conditioning are two important aspects of the study of the effects of weightlessness in the neural control of cardiovascular functions. Also the study of periodicity, baseline shifts, effects of feeding and physiological stimulation should be included. Neurographic analysis of controlling neurophysiological mechanism should also be considered.

The individual investigations reported in this final progress report illustrates a number of experiments which could be used as models to determine the possible effects of prolonged weightlessness on the neural control of cardiovascular functions. The practical application of these findings will be possible when the adapting psychocardiovascular mechanisms under weightlessness are understood and therapeutic measures are taken to enhance or control them.

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LABORATORY ENVIRONMENTAL CONDITIONS

The laboratory facilities were located in the Pavlovian Laboratory at the Johns Hopkins University School of Medicine.

Figure 1 illustrates the one-way window on the sound proof room (Industrial Acoustics Company, New York). The recording equipment on the right is an Offner transistorized polygraph.

Figure 2 illustrates the programming and monitoring equipment which was used in the various experiments reported herein.

Psychocardiovascular reactions were studied in monkeys restrained in plastic monkey chairs. Figure 3 shows a restrained primate. Note the wide section in the chair showing the monkey's abdominal region. In this preparation we measured heart rate, electrocardiograms, blood pressure and blood flow without disturbing the animal. Figure 4 is a close-up picture of the restrained primate. A similar restraining device could be modified for space flights. Figure 5 shows the restrained primate inside the sound proof room.

In our studies we projected that eventually we would prefer to work with the unrestrained primate. This preparation could be trained to do various behavioral tasks without being restrained in a monkey chair. Telemetry techniques were planned as a possible solution and we have tried some of these techniques.

In the unrestrained primate we have solved some problems such as preventing the animals from tearing wires and expensive transducers. The first step was to develop a suit or biological casting which the primates could ware. Figure 6 illustrates one suit that showed some promise along these lines. Monkeys were dressed in this suit with a protective helmet. This combination of suit and helmet allowed the primates to walk freely without tearing valuable transducers. Figure 7 illustrates a primate wearing this space suit.

Experimentation in restraining devices which could be transported easily were also developed as shown in Figure 8. This proto-type movable unit could be transported easily from laboratory to laboratory or from the laboratory to the launching site without excessive attention by paramedical personnel. A similar unit of lighter weight could be used as a ready assembly package with life support system accessories for possible placement in a live launching unit.

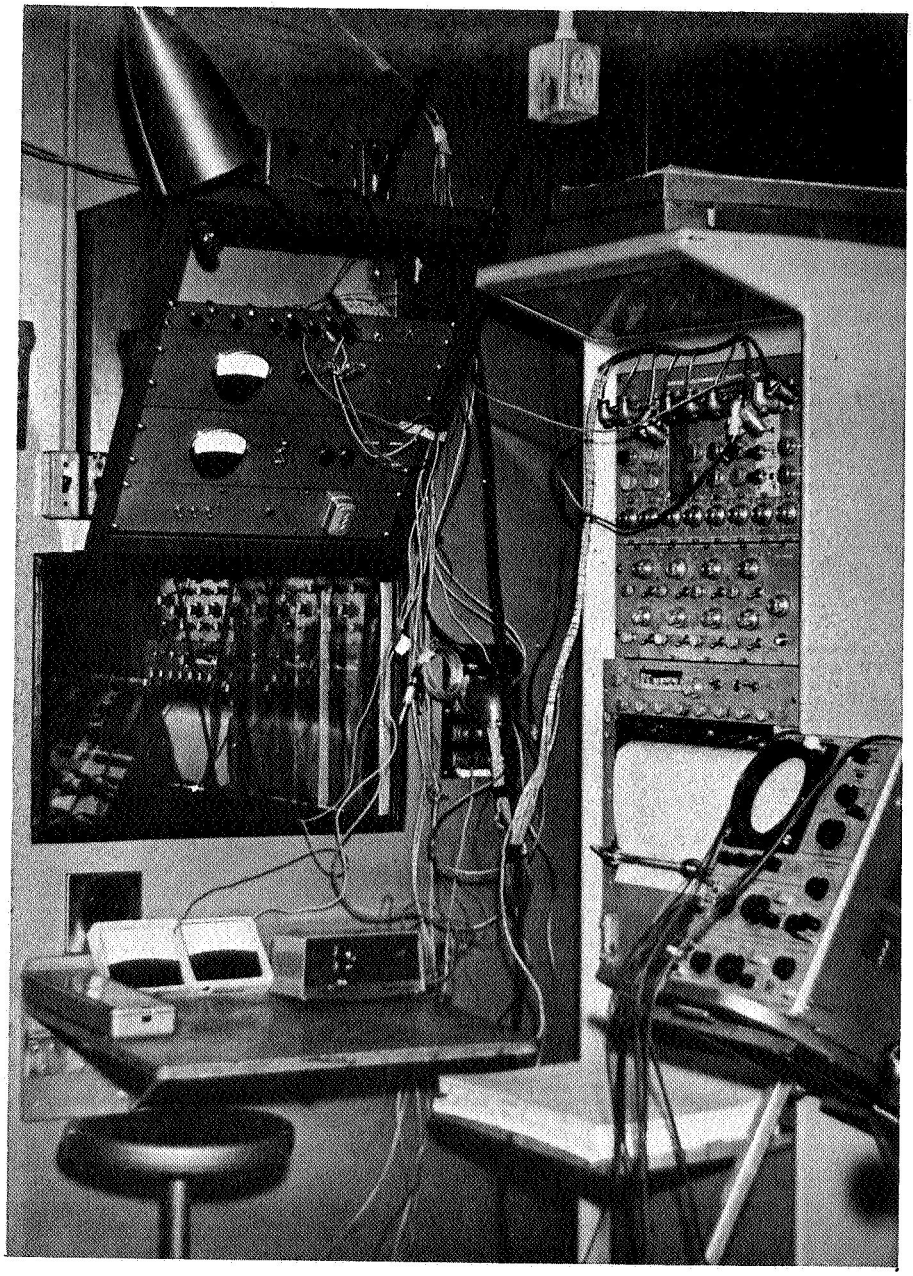


Fig. 1.

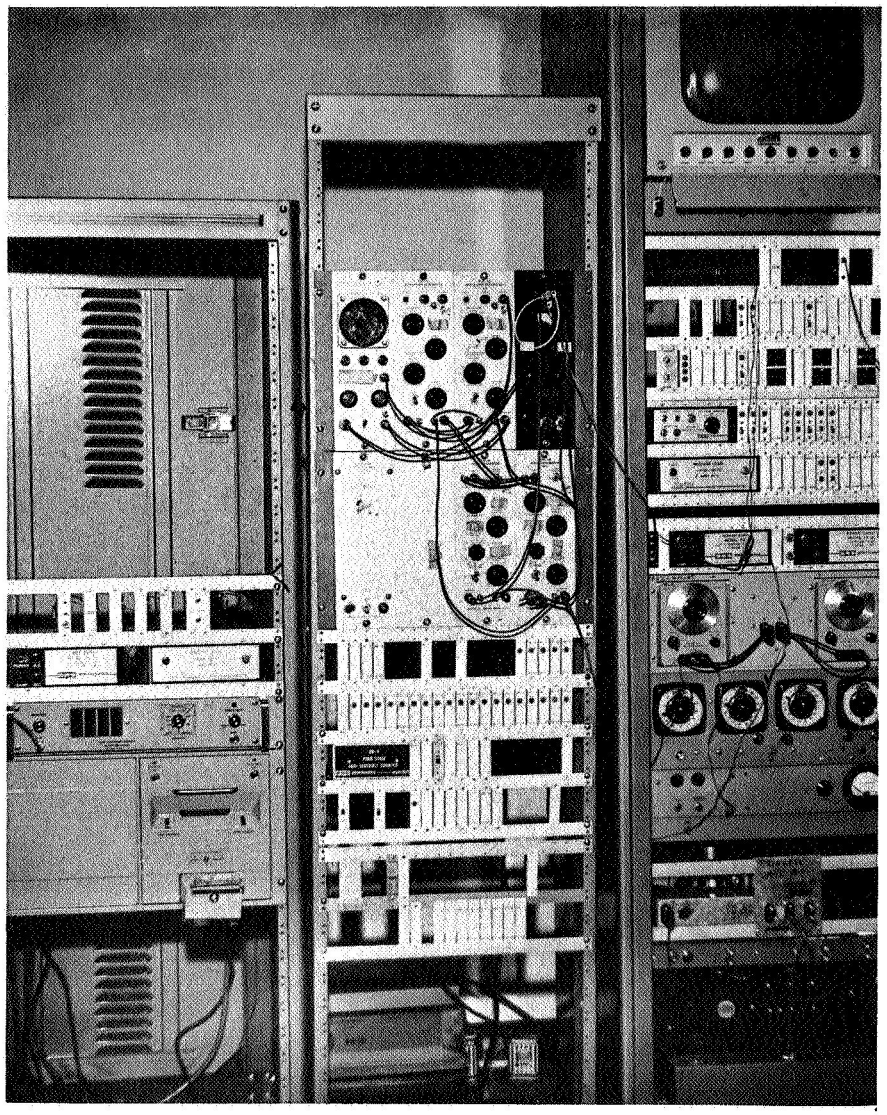


Fig. 2.

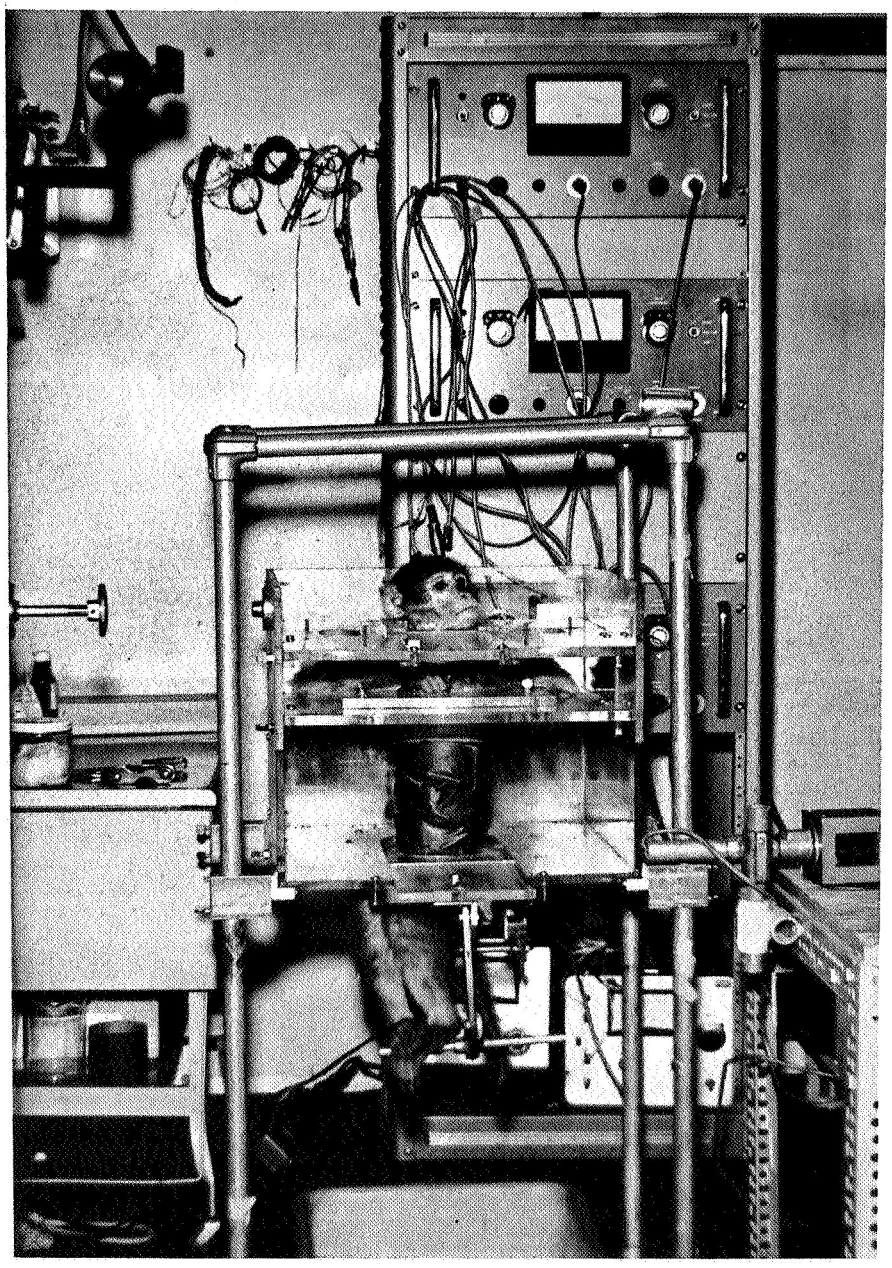


Fig. 3.

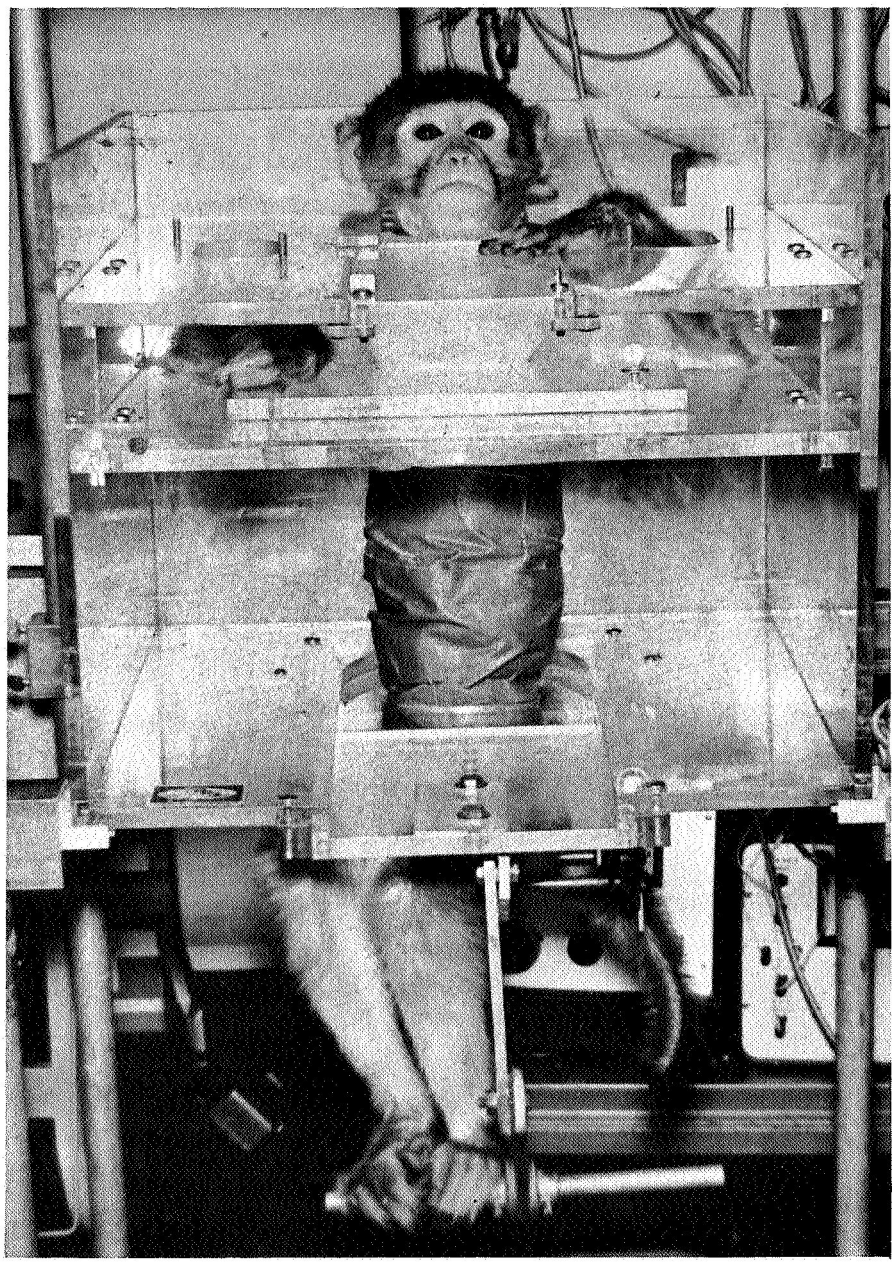


Fig. 4.

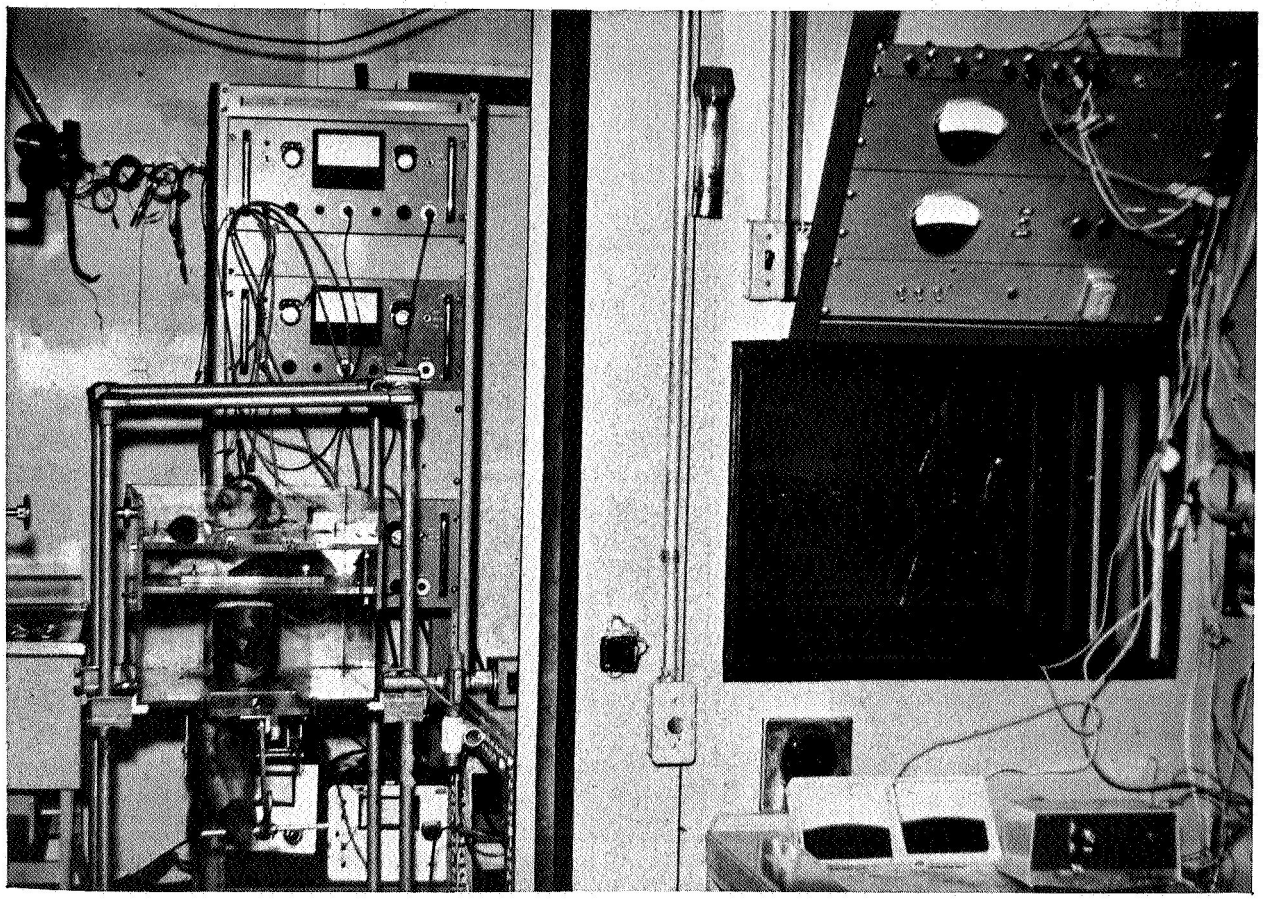
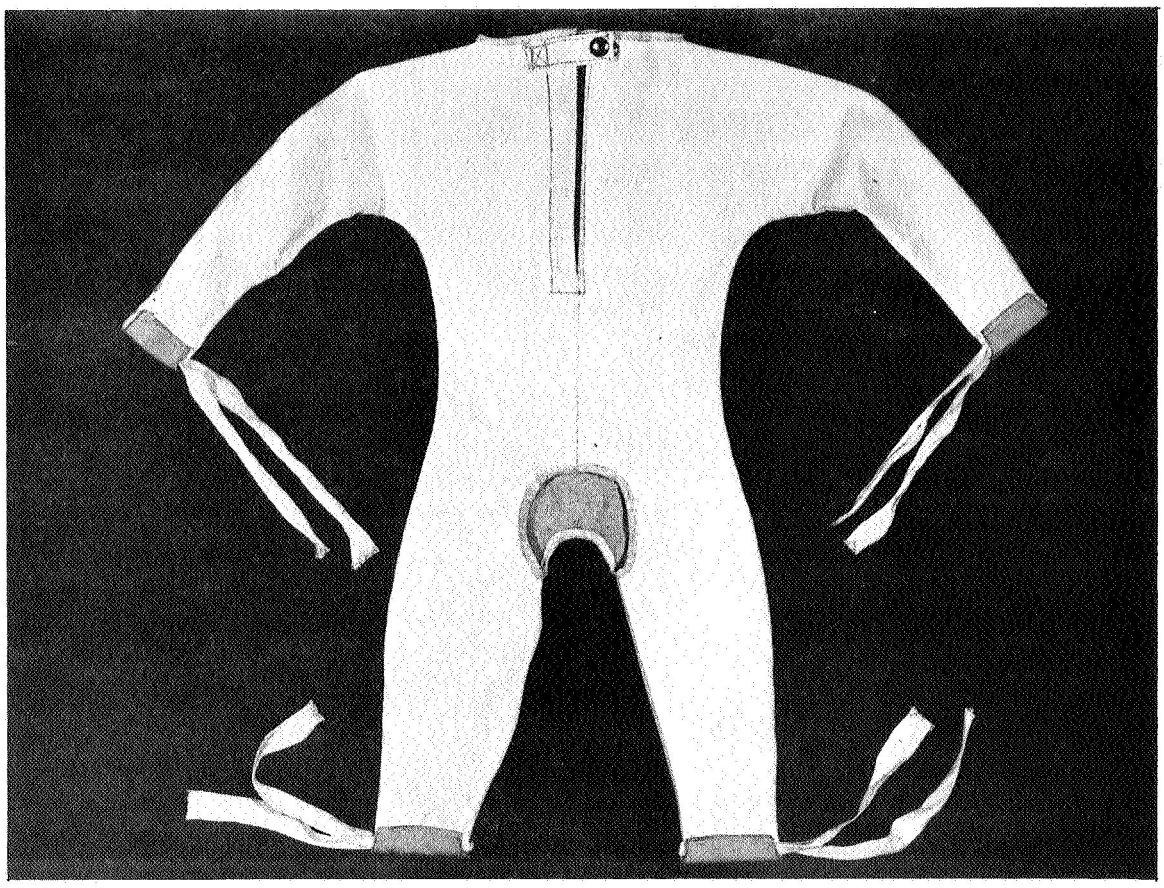


Fig. 5.



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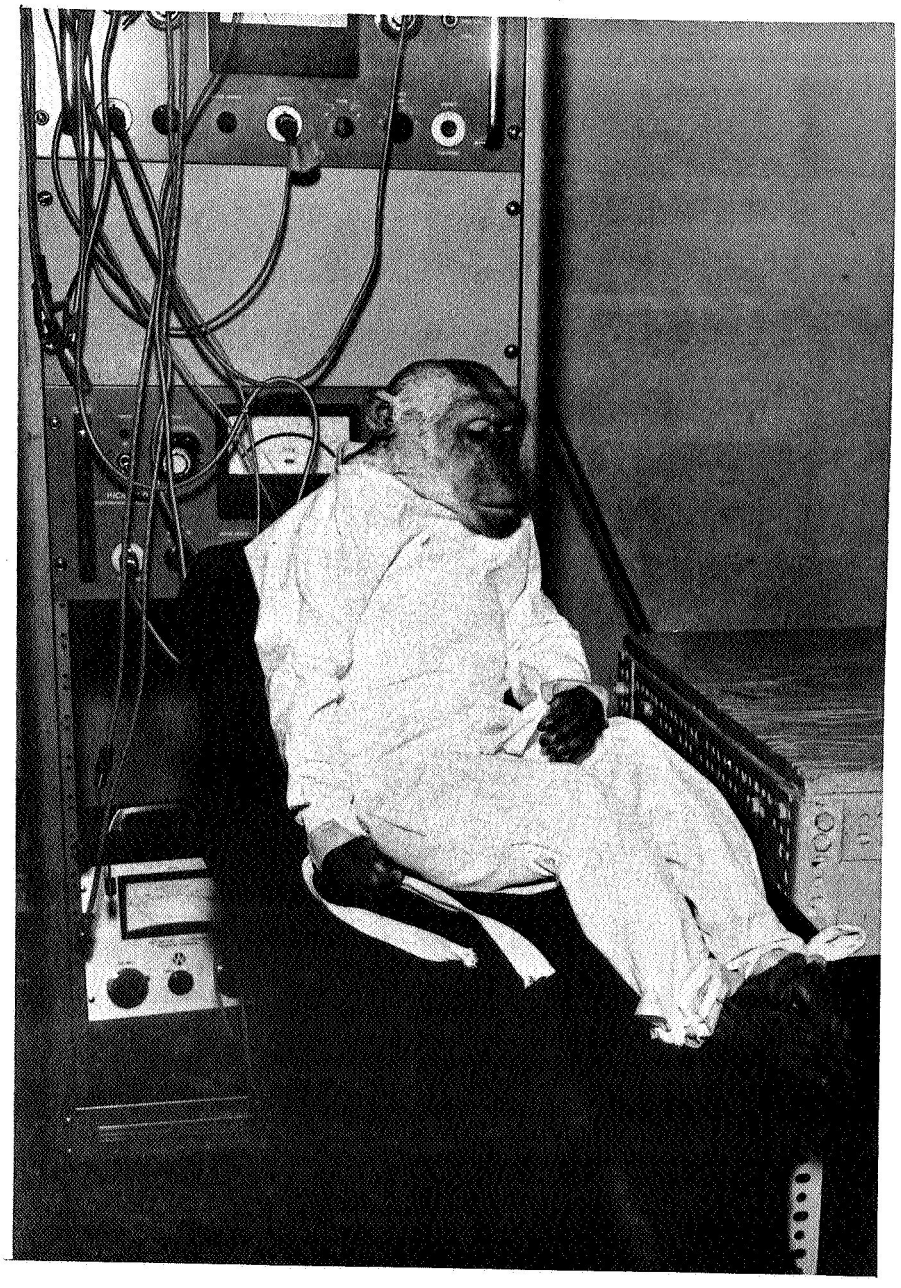

Fig. 7.

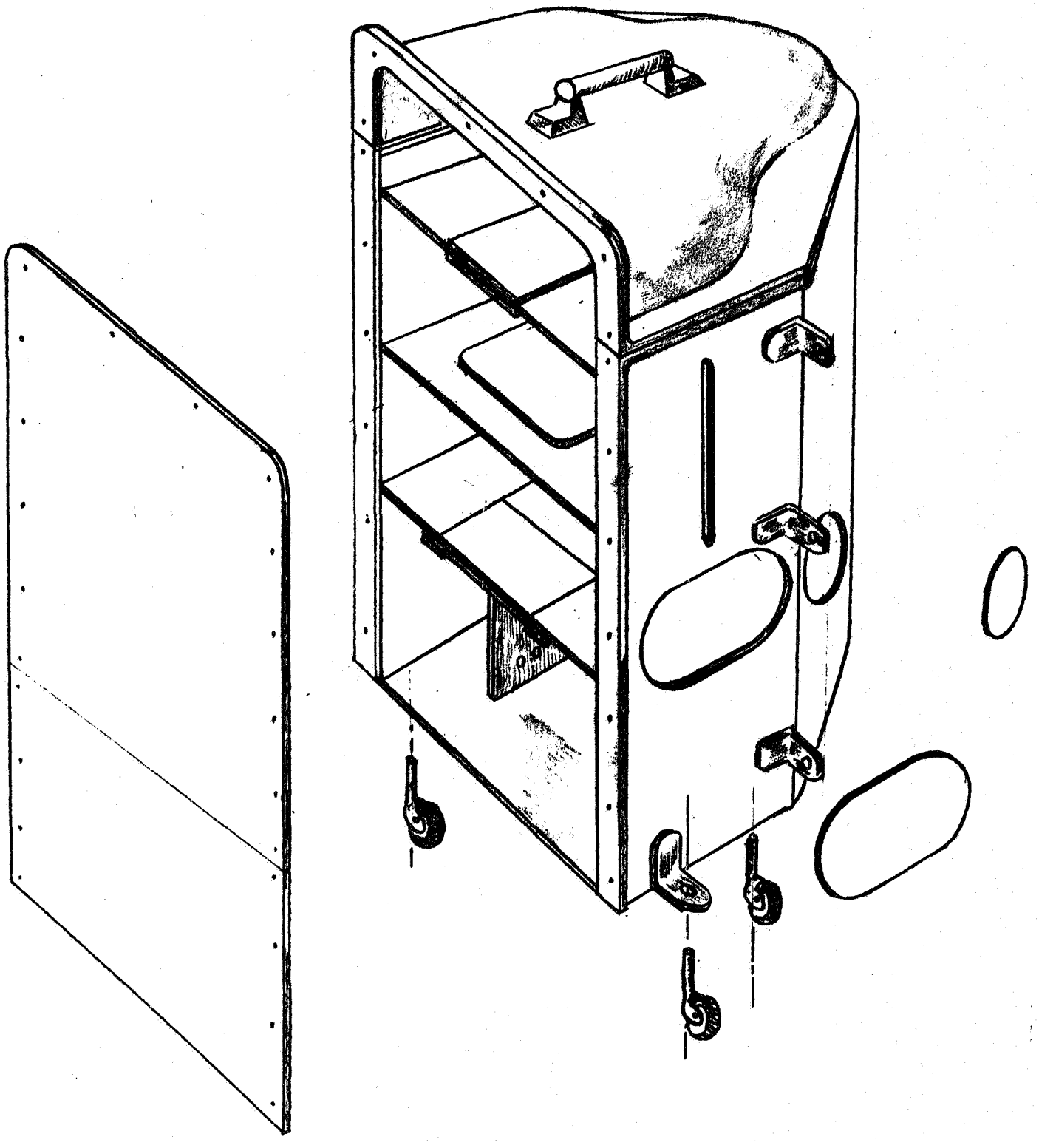

Fig. 8.

PHYSIOLOGICAL MEDIATION OF THE CARDIOVASCULAR ORIENTING REFLEX IN DOGS.

The purpose of the present study is to analyze the HR changes beat-to-beat during the first trial in which a novel stimulus is presented, and to analyze the various neurological, muscular and respiratory mechanisms which might influence this response.

A number of investigations have shown that the usual initial cardiac OR is a bradycardia which changes in many instances to a tachycardia after a few presentations of the novel stimulus (Graham and Clifton, 1966, Black, 1964; Stern & Wood, 1961; Lynch, 1967; Newton and Perez-Cruet, 1967). In general these studies have used a trial by trial analysis of HR changes.

Although cardiac changes as a component of the orienting response were first elaborated by Robinson and Gantt (1947) and the pattern of the HR-OR over a number of trials well documented, the OR within the first trial has not been clearly analyzed, nor has the physiological mediation of this response been defined. In studies by Lynch (1967) evidence was presented showing that the HR-OR could function independent of respiratory changes, which led to the suggestion that the HR-OR was centrally mediated. Perez-Cruet and Gaertner (1967) noted that the HR slowing during OR is not necessarily a parasympathetic response, since this HR bradycardia was observed in dogs bilaterally vagotomized. These observations lead to the question of what are the mechanisms that control the HR-OR. The present study represents an attempt to define the relative influence of the parasympathetic, the sympathetic, muscular, respiratory and baroreceptor influences on the HR-OR. This analysis was attempted by both surgical and pharmacological blockade of the various physiological mediators in a large group of dogs.

Procedure:

The subjects were 49 experimentally naive, mongrel dogs. The subjects were placed in six major groups as follows:

1. Normal control group
2. Bilaterally sympathectomized group
3. Bilaterally vagotomized group
4. Parasympathetic blockade with atropine
5. Beta adrenergic blockade with propranolol
6. Muscular paralysis with succinylcholine artificial respiration

All the animals were placed in a sound-proof, temperature controlled room, and observed through a one-way mirror and television monitor. (Fig. 1, See appendix for slide proofs). Electrocardiograms and respiration were measured with an Offner polygraph. In several of the dogs arterial blood pressure, electromyograms from the neck and hindlegs were measured simultaneously with heart rate. Beat-to-beat HR was analyzed with a Hewlett-Packard electronic counter, and then programmed into a computer.

The orienting stimulus consisted of a 60 db tone, 480 cps, presented for 10 seconds. The first tone was presented 4-5 minutes after the animal was settled in the experimental room.

Surgical Procedure: Bilateral cervical vagotomies were performed in 12 dogs with a technique recently developed by Perez-Cruet and Gaertner (In Press). Resection of the vagosympathetic trunk in the neck included about 1 to 2 inches of the cervical vagi. Complete bilateral vagotomies were verified by a number of physiological changes such as elevated heart rate, vomiting, relaxed nictitating membranes while the animals were alive, and at autopsy.

Bilateral sympathectomies were performed by removing the stellate ganglion and also the thoracic sympathetic chain up to the 10th thoracic intercostal space. Signs of sympathetic resection included relaxed nictitating membranes, prominent sinus arrhythmia and slow heart rate.

Drug Procedure: Pharmacological denervation was done with atropine (dose: 0.3 mg/Kg, subcutaneously)to block the parasympathetic system and with propranolol (Inderal dose: 5 mg/Kg, intravenously) to block the beta adrenergic mechanisms of the sympathetic nervous system. Signs of atropinization consisted of dry mouth, and very elevated heart rate. The beta blockade with inderal was complete and the response to isoproterenol was obliterated.

In order to rule out the mediation of the orienting bradycardia by muscular or respiratory factors a group of animals were paralyzed temporarily with succinylcholine (dose: 1 to 2 mg/Kg, intravenously) and ventilated artificially. Electromyogram showed no evidence of muscular activity during paralysis by succinylcholine.

Results:

The summary results are presented in Table I. As shown in Table I 67% of the normal control dogs showed an orienting bradycardia in the first orienting trial; 25% showed no change in heart rate and only 8% acceleration. Slide I illustrates in the normal controls average changes in beat-to-beat heart rate changes before, during and after the first orienting trial. Note that initially there is a sudden drop in heart rate followed by a more gradual slowing. Similarly when the orienting stimulus is terminated there is another initial sudden drop in heart rate followed by an increase in heart rate but still below the pre-stimulus level.

In the vagotomized group, 50% showed a gradual deceleration of about 5 to 10 beats per minute during the orienting stimulus; 33% showed no change and 17% accelerated (see Table I). The overall average for this group is shown in slide 2.

In the atropine group, 60% showed complete obliteration of the orienting bradycardia; 20% showed bradycardia and 20% accelerated (See Table I). The overall average is shown in slide 3.

In the Inderal group, 90% showed definite orienting bradycardia with very pronounced and accentuated initial sudden drops in heart rate; none developed acceleration and 10% showed no change. The overall average for the group is shown in slide 4.

In the sympathectomized group, 50% developed orienting bradycardia; none developed acceleration in heart rate and 50% showed no change. The overall average for the group is shown in slide 5.

In the curare group, 33% showed acceleration and 17% showed bradycardia, while 50% showed no change. The overall average for this group is shown in slide 6.

Implications and Conclusions:

This study confirms that an initial slowing of heart rate occurs during the first orienting trials.

The mediation of this bradycardia is not entirely vagal since it occurs in dogs with bilateral cervical vagotomies. Rosenblueth and Freeman (1931) have found that slowing of the heart rate with sympathetic nerves intact is possible in vagotomized anesthetized cats. In their experiments stimulation of the central afferent stump of the vagus nerve with bilateral cervical vagotomies produced heart rate slowing. This finding by Rosenblueth, et al, and our findings of slowing of heart rate in the unanesthetized vagotomized dog suggest that the orienting bradycardia can in fact be mediated by inhibition of the sympathetic nervous system. The fact that slowing of heart rate occurs in the vagotomized dog during orienting also suggests feedbacks between vagal excitation and sympathetic inhibition.

The finding that the orienting bradycardia was affected more by atropine than by vagotomy suggest that this drug not only interferes with parasympathetic influences on the heart, but also has known undesirable central effects.

The results in the propranolol and sympathectomized groups suggest that the neurological mediation of the acceleration in heart rate as a component of the orienting reflex is by means of sympathetic excitation. This fact is supported by the finding that not a single dog in these two groups showed acceleration but rather a consistent deceleration as an orienting reflex. The results also suggest that this acceleration is not mediated by parasympathetic inhibition because after symphatectomy or beta adrenergic blockade, where parasympathetic influences are not blocked, not a single case of acceleration as an orienting reflex was observed during the first orienting trial. The fact that bradycardia as a component of the orienting reflex was more prominent in these two groups also suggest that excitation of the parasympathetic system is an important mediator of the orienting bradycardia.

The curare experiments showed definite evidence of orienting bradycardia in one animal, but 2 others showed acceleration while the others did not show any heart rate response. These findings suggest that at least in some animals complete muscular paralysis and controlled respiration cannot obliterate the acceleration component of the orienting reflex, but that it does affect greatly the bradycardia component. However the presence of one instance of orienting bradycardia in dogs paralized temporarily with succinylcholine clearly illustrate that the bradycardia can be central in origin without intervening muscular mediation.

Measurement of blood pressure showed a decrease in systolic and diastolic blood pressure which indicate that the orienting bradycardia is not mediated by baroreceptor mechanisms.

Electromyograms from the neck and hindlegs usually showed a reduction in the amplitude of muscle potentials in the majority of cases, however, some dogs showed an increase in muscle potentials during orienting bradycardia indicating that the musculo-skeletal mediation mechanism is not always responsible for the cardiac effects.

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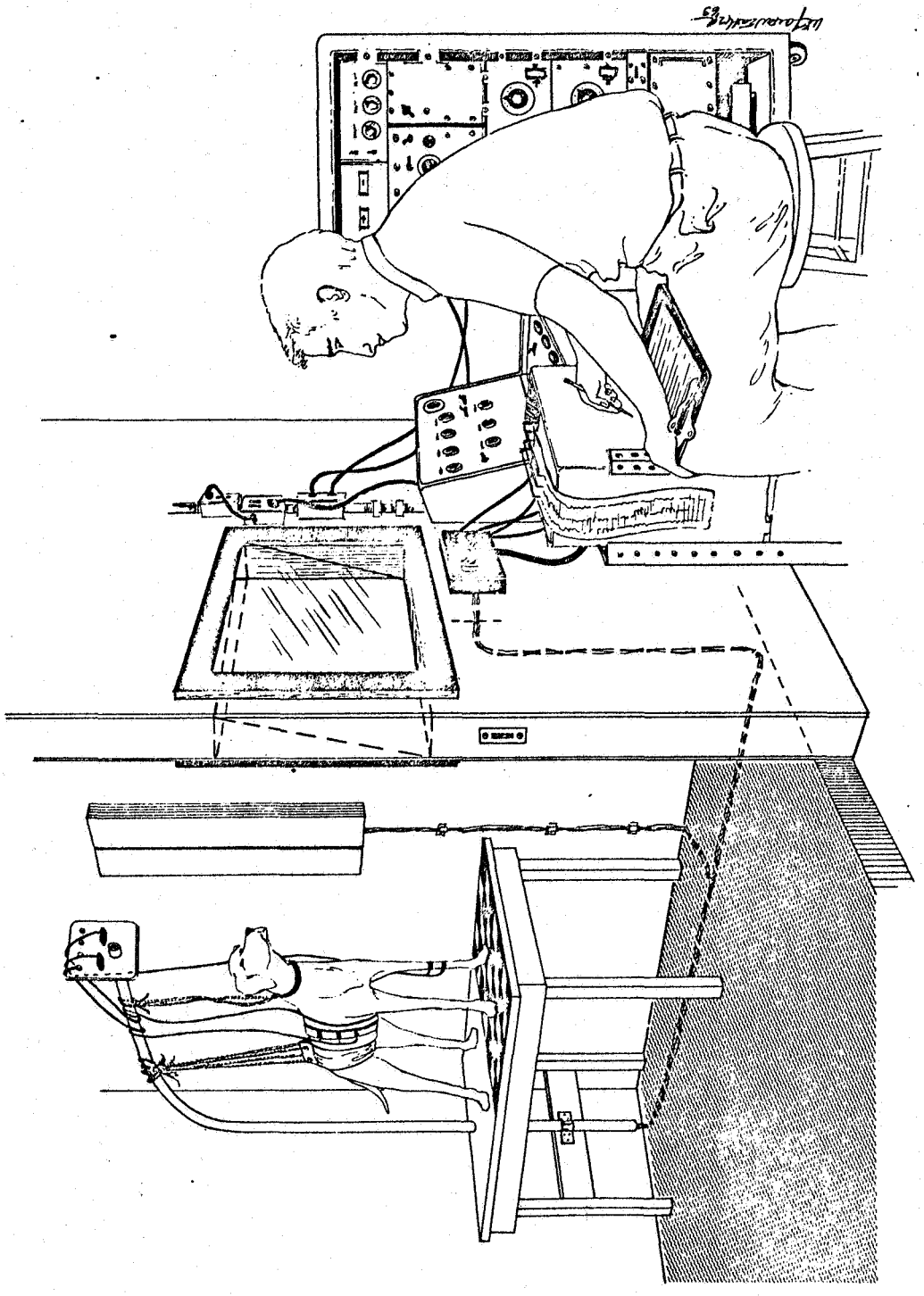
TABLE I

HEART RATE CHANGE AS A COMPONENT OF THE ORIENTING REFLEX

IN THE FIRST ORIENTING TRIAL

	During Tone		
	Acceleration	Deceleration	No Change
Normals	8%	67%	25%
(Dogs)	1	8	3
Vagotomies	17%	50%	33%
(Dogs)	2	6	4
Atropine	20%	20%	60%
(Dogs)	1	1	3
Sympathectomy	0%	50%	50%
(Dogs)	0	2	2
Inderal B-blockade	0%	90%	10%
(Dogs)	0	9	1
Curare	33%	17%	50%
(Dogs)	2	1	3

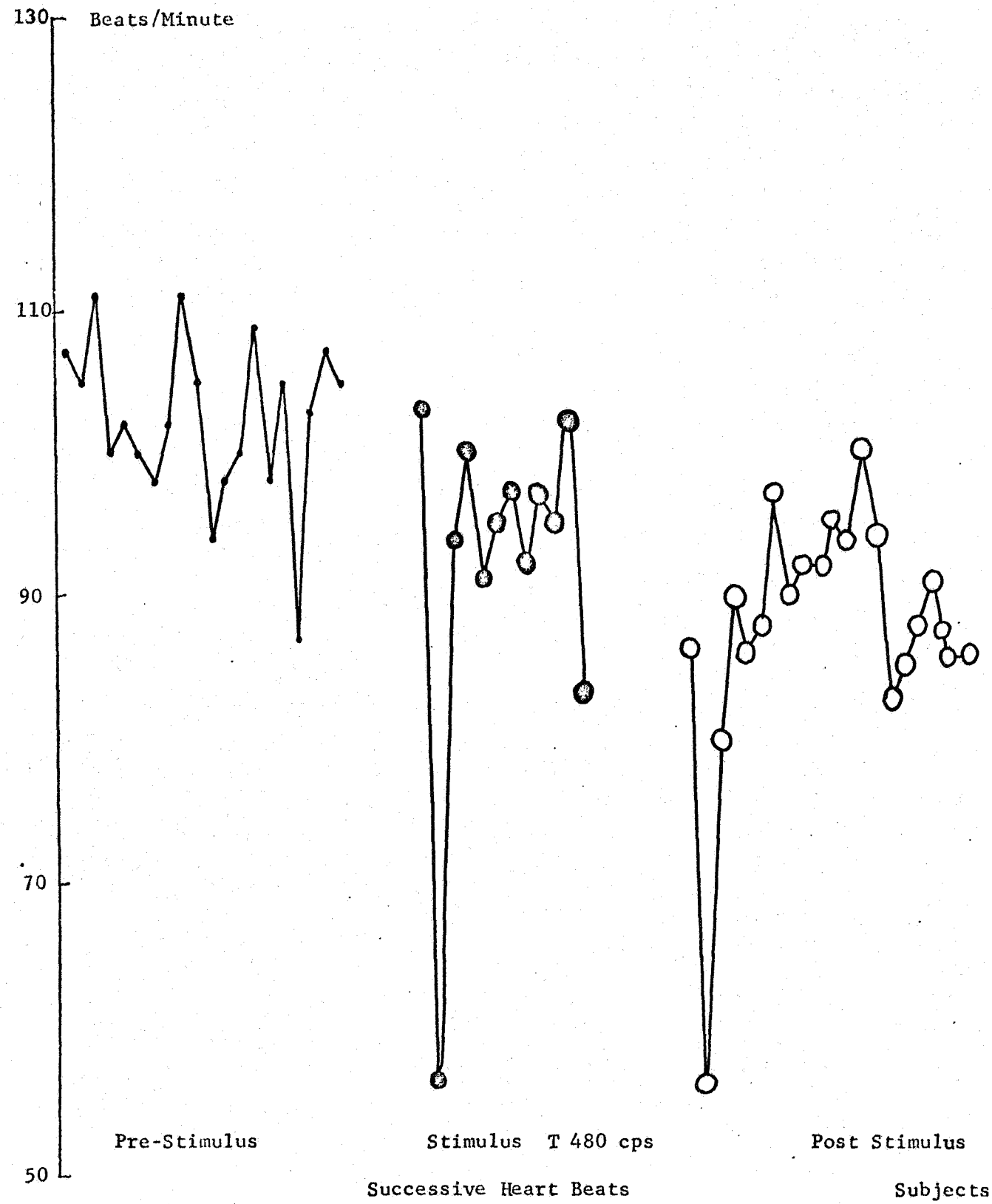


PAVLOVIAN CAMERA

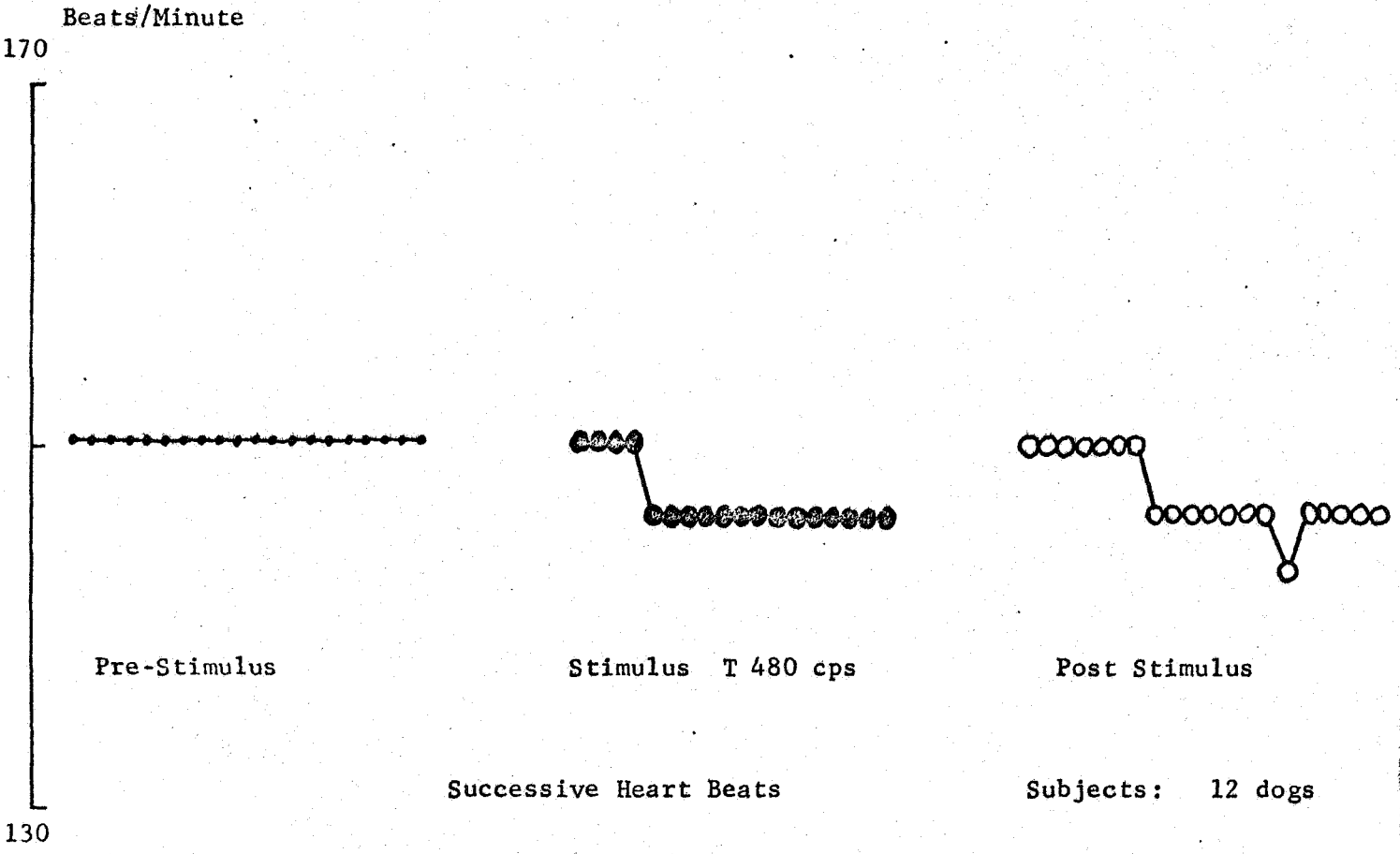
FIG. 1

SLIDE # 1

CONTROL GROUP: ORIENTING HEART RATE SLOWING DURING FIRST ORIENTING TRIAL



EFFECT OF BILATERAL CERVICAL VAGOTOMY ON THE HEART RATE COMPONENT OF THE ORIENTING REFLEX



SLIDE # 2

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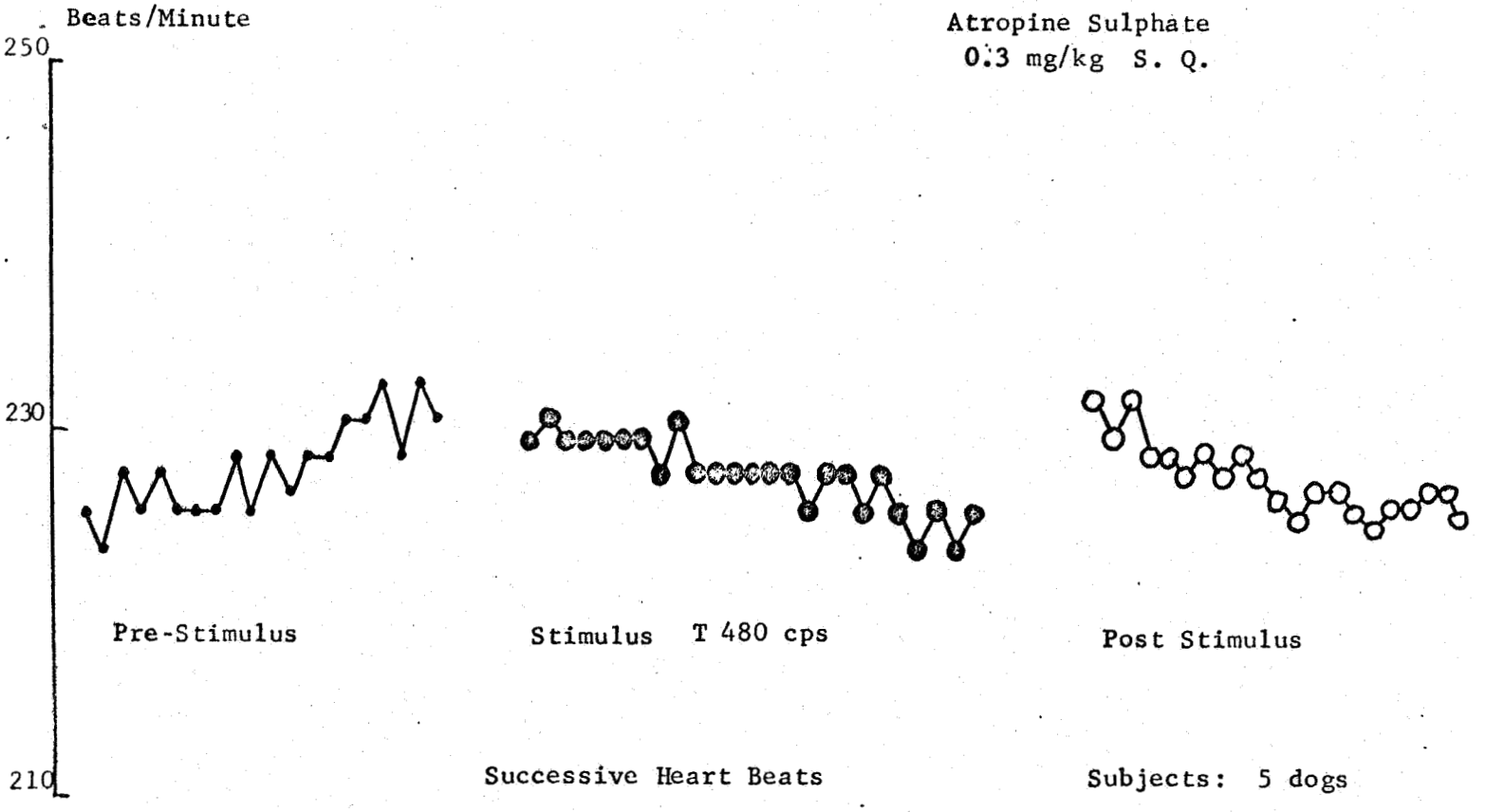
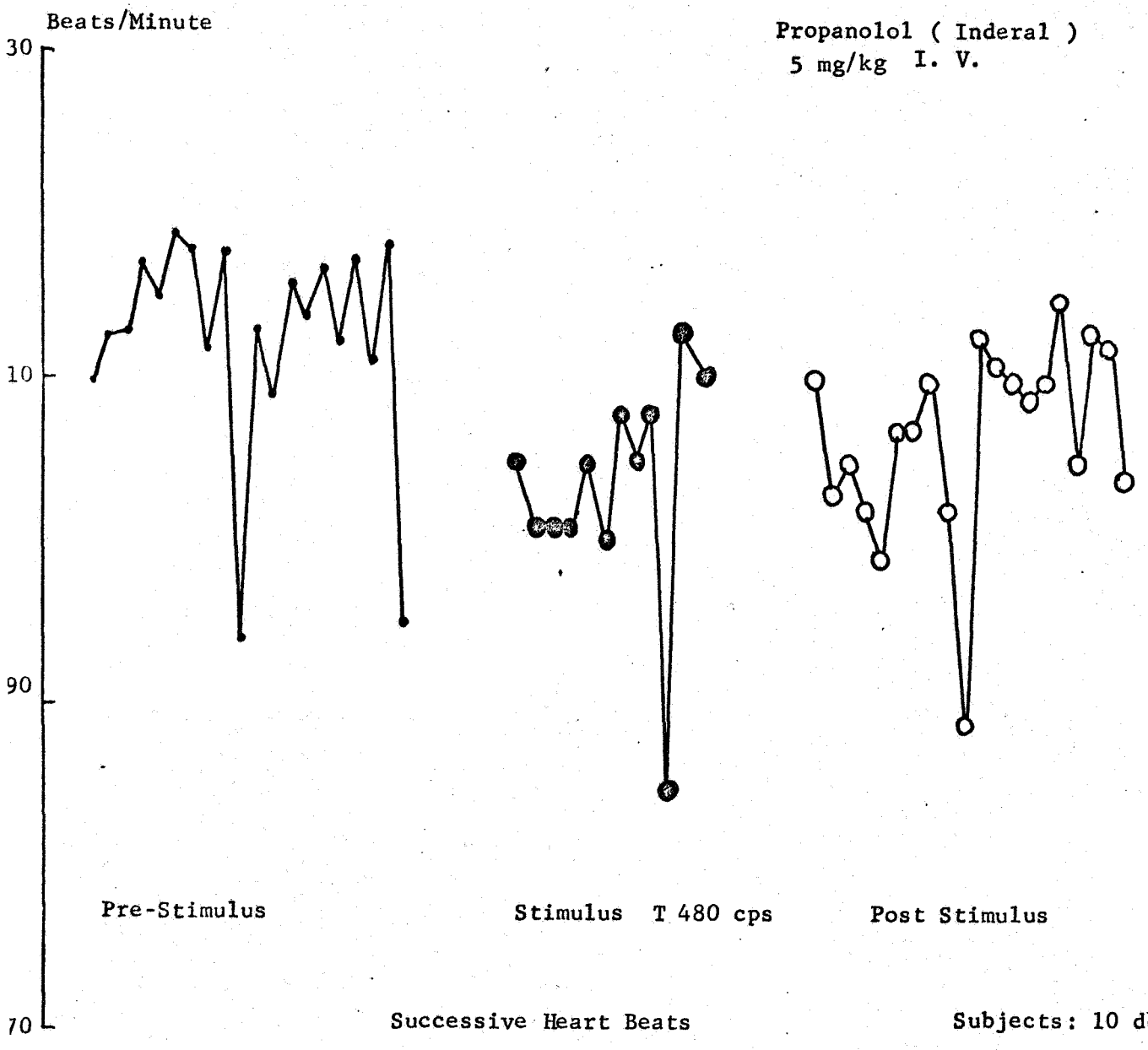
EFFECT OF ATROPINE ON THE HEART RATE COMPONENT OF THE ORIENTING REFLEX



SLIDE # 4

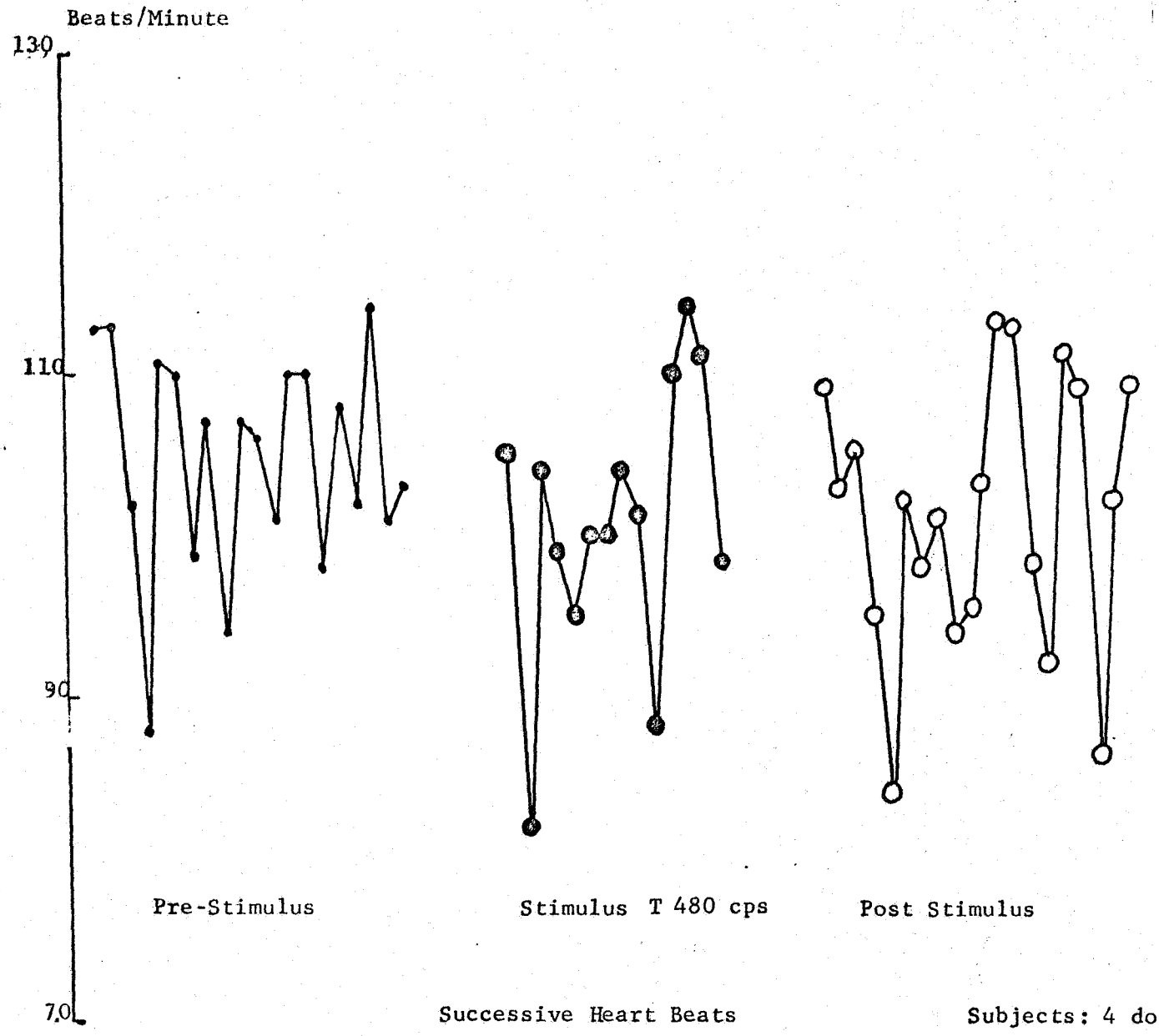
EFFECT OF BETA ADRENERGIC BLOCKADE ON THE HEART RATE COMPONENT OF THE ORIENTING REFLEX



Subjects: 10 dogs.

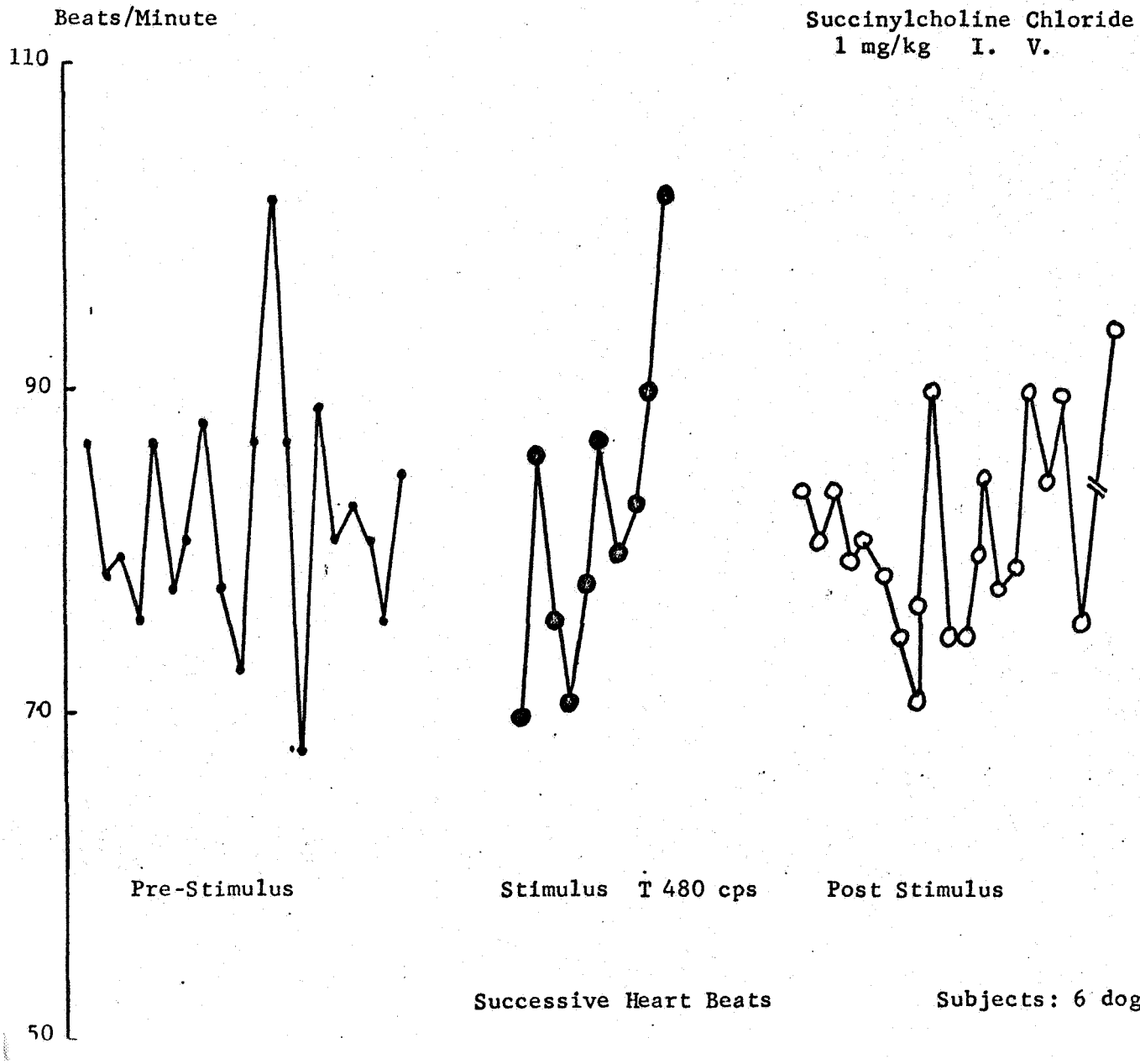
SLIDE # 5

EFFECT OF SYMPATHECTOMY ON THE HEART RATE COMPONENT OF THE ORIENTING REFLEX



SLIDE # 6

EFFECT OF SUCCINYLCHOLINE ON THE HEART RATE COMPONENT OF THE ORIENTING REFLEX



HEART-RATE DURING ORIENTING AND CLASSICAL DEFENSIVE CONDITIONING IN MONKEYS.

There are several reports concerning changes in heart rate (HR) during classical defensive conditioning in humans[1,2], and dogs[3], and other species[4]. On reviewing the literature, we have not found studies concerning the cardiovascular components of the orienting reflex in primates.

Smith and Stebbins[5] have studied blood flow from the lower aorta and HR in primates, which were isolated in a quiet room. In their studies, they used delayed classical conditioning with two different lights as conditional stimuli. One light was paired with shock and the other was non-reinforced. They found changes in HR and blood flow during conditioning and they ruled out that body movements and respiration were responsible for the conditional changes.

The purpose of the present study was to determine if HR CRs could be established in monkeys using a paradigm which has been employed in our laboratory for conditioning in dogs[6].

Methods and Materials.

Two monkeys were used. The animals were isolated in a soundproof room with a sound attentuation of 81 db, and they were observed throug a one-way window. The animals were restrained in monkey chairs and all physiological measurements were done from outside of the room.

The EKG was obtained with implanted electrodes under the skin. One electrode was implanted in the right side of the thorax and the other was implanted in the left inguinal region. This lead recording is similar to the standard lead 2, commonly used in electrocardiography. A ground electrode was usually implanted in the left side of the thorax. The HR changes during orienting and conditioning were obtained by measuring manually the R-R interval (RRI) between successive R waves in the EKG. The HR was also monitored with a Gilford cardiotachometer which displayed a beat-by-beat change in HR.

The technique for evaluating the HR changes during orienting and conditioning by successive beats has been described recently by Newton & Perez-Cruet.[7] In this technique, the HR is averaged beat-by-beat before, during and after a tone. During analysis, all RRI for several trials for the last RRI prior to stimulus onset (RRI -1) are averaged and the standard deviation is computed. Similarly, working backward in time, the same standard deviation is computed. Similarly, working backward in time, the same procedures are carried out for RRI -2 (next to last RRI before stimulus onset) and so on until RRI -10. Starting again at tone onset, the average HR and the standard deviation are computed for RRI +1 up to RRI +20 during the tone. At the tone offset, averaging is begun again working forward for 10 to 15 RRI. This type of analysis gives a picture of the latency of the response and the magnitude of the changes. The orienting reflex was studied with techniques employed previously in our laboratory by Robinson and Gantt[8]. The orienting training consisted of presentation of tones which were later to become the CS. Tones 256 cy/sec. and 512 cy/sec. were presented alternately at 2 minute intervals for 10 to 30 trials of each tone per day. The duration of the tone was 8 seconds. The conditioning training was done after 50 to 100 orienting trials of each tone (2 to 3 days). During conditioning, tone 256 cps was always reinforced with a mild shock (5-10 volts and less than 5 ma. with a duration of ½ sec.), just sufficient to cause a withdrawal of the right leg. The

electric shock was always applied over the skin on the right leg. Tone 512 was never reinforced. The intertrial interval during conditioning was varied between 1 to 2 minutes. There were 50 to 100 reinforced trials of conditioning per monkey (several daily sessions). The reinforced tones were called the excitatory tones and the non-reinforced were called the inhibitory tones.

Results:

A monkey (F309) showed significant increases in HR to tones 256 and 512 during orienting. The increase in HR during orienting varied from a baseline of 191 to a peak value of 204 bpm during tone 512; and from a baseline of 196 to a peak value of 205 bpm during tone 256. In another monkey (C64) changes in HR during orienting were less pronounced and there was no significant changes in the HR component of the OR during tones 256 and 512. During conditioning the two monkeys showed a significant acceleration in HR to the excitatory tone 256 cps. With our procedure there was no evidence of clear cut differentiation between tones 256 and 512, but the changes in HR during the excitatory tone were greater than those during the inhibitory tone in monkey C64.

Figure 1 and 2 illustrate the changes in HR during orienting (A) and conditioning (B) to tones 256 and 512 in monkey C64. Note that during orienting the HR does not change significantly during the tones. However, during conditioning, tracing B, there is a gradual increase in HR after the 5th beat and a large increase in HR after the 15th beat during tone 256, showing a HR conditional reflex from 174 bpm (baseline) to 201 bpm. The unconditional stimulus (US) which uses a shock to the right leg, produces a slight increase in HR (see Figure 1). During the non-reinforced tone 512, there is also an increase in HR from a baseline of 176 to a peak value of 190 bpm during the tone.

Figures 3 and 4 illustrate changes in HR during orienting and conditioning to tones 256 and 512 in monkey F309. Note that the changes in HR during orienting are similar to the changes in HR during conditioning.

In monkey C64, the conditioning training produced a lower HR baseline than that observed during orienting; on the other hand, in monkey F309 the conditioning training produced an elevation of the HR baseline from that observed during the orienting training. Similar results have been observed in HR conditioning in dogs.

Inhibitory cardiac conditional reflexes were occasionally seen in monkey F309. The cardiac inhibitory conditional reflex was observed during and after the non-reinforced tone and it consisted of a HR deceleration. This HR deceleration became prominent at the offset of the tone. A tracing showing an inhibitory cardiac CR is shown in Figure 5.

Discussion

This preliminary study shows that the HR conditional reflexes in the two monkeys consists of an acceleration similar to that seen in dogs. The HR acceleration as a component of the orienting reflex can also occur in monkeys. Differentiation in these two monkeys was not as good as in dogs.

The studies of Smith & Stebbins has shown discrimination of HR in 3 out of 4 monkeys. In their studies, they used a conditional stimulus of 56 sec. with varying intertrial intervals of 5, 10 and 15 min. and a time between sessions of at least 48 hours. This difference in procedure might account for the good differentiation observed in their experiments. In the present study, we were attempting a comparison of the HR conditional reflexes in monkeys versus dogs using the same paradigm for both animals. In spite of the difference of methods from Smith & Stebbins, we also found the HR CR to be accelerative.

The fact that inhibitory cardiac CR to the non-reinforced tone was observed in one monkey indicates that the HR deceleration can also be used as an index of inhibition as previously shown in dogs by Perez-Cruet[9]. Studies are now in progress to determine other cardiovascular functions during orienting and conditioning in primates.

Summary

Heart-rate changes during orienting and conditioning were studied in two monkeys. A heart-rate acceleration as a component of the orienting reflex was observed in one monkey. Heart-rate acceleration as a conditional reflex was observed in two monkeys. Differentiation between a reinforced excitatory tone and a non-reinforced inhibitory tone was not observed. HR deceleration during the non-reinforced tone was occasionally observed in one monkey.

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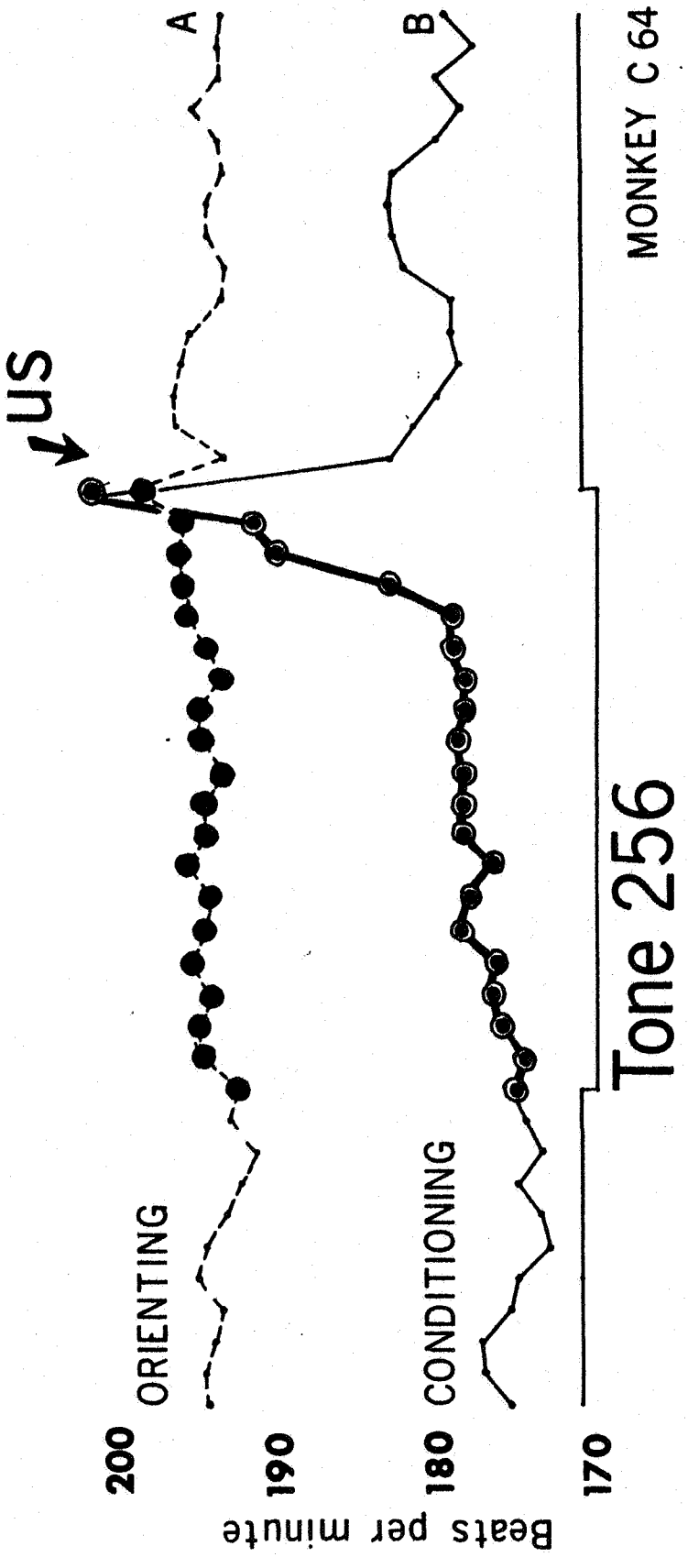
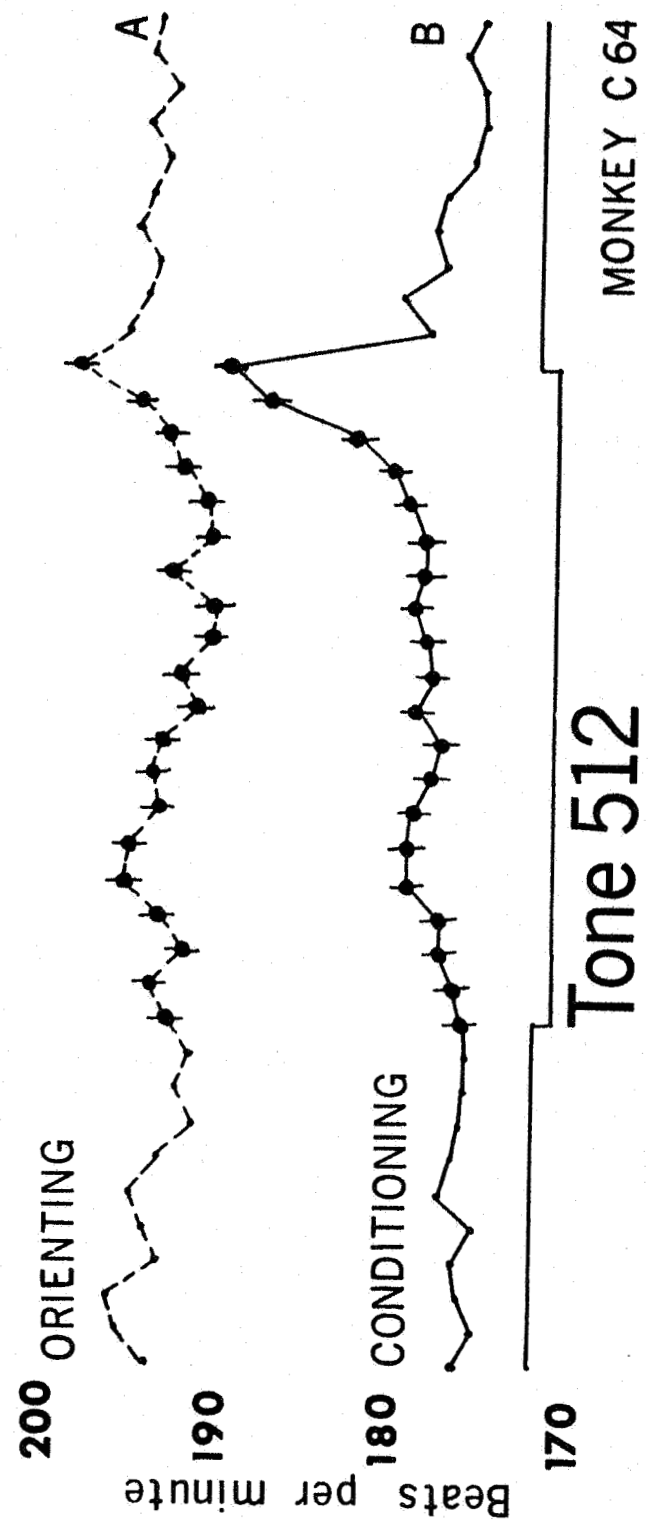

Beats per minute
200
190
180
170
ORIENTING
CONDITIONING
US
A
B
Tone 256
MONKEY C 64

Fig. 1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Beats per minute
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MONKEY C64

Fig.2

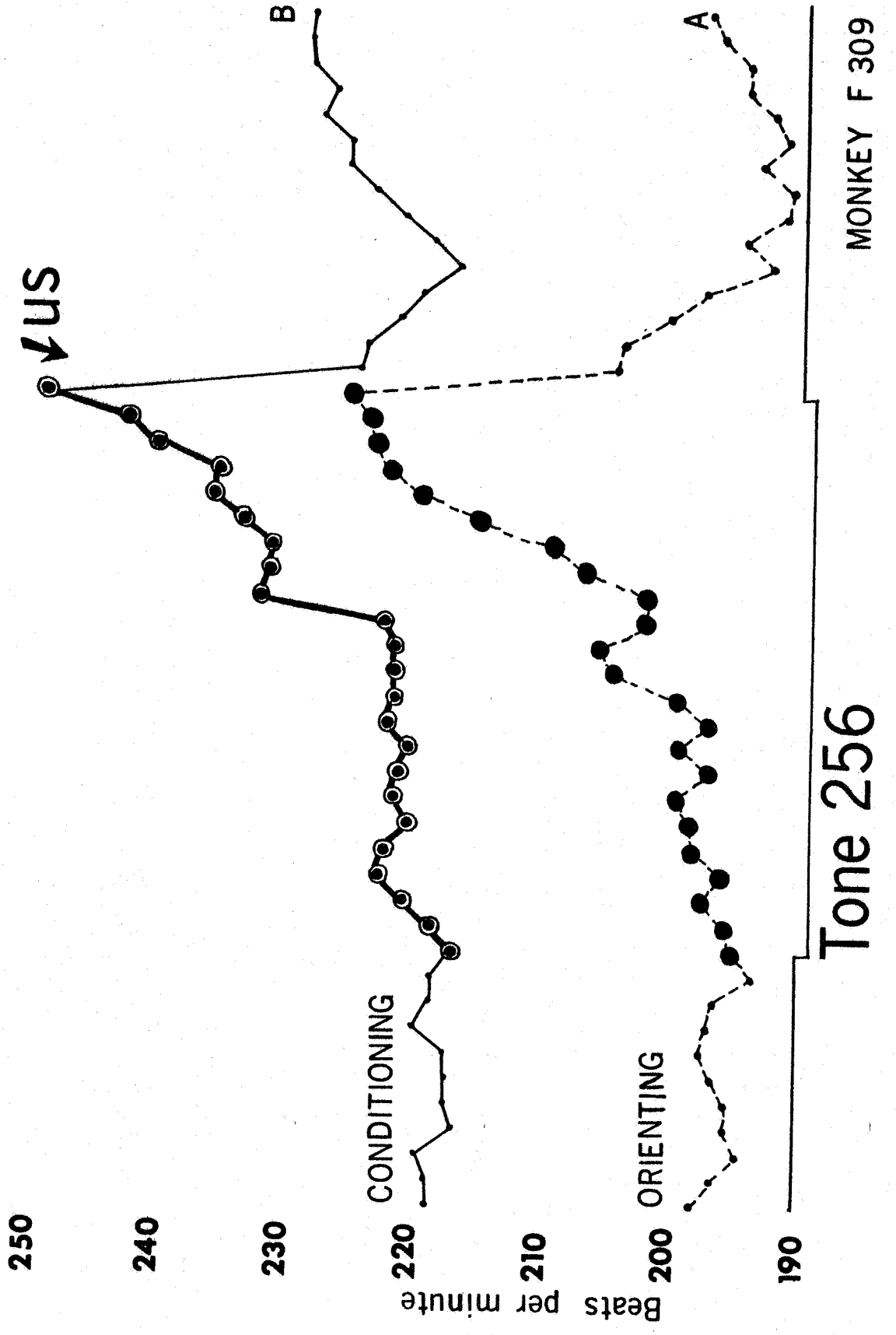
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Fig. 3

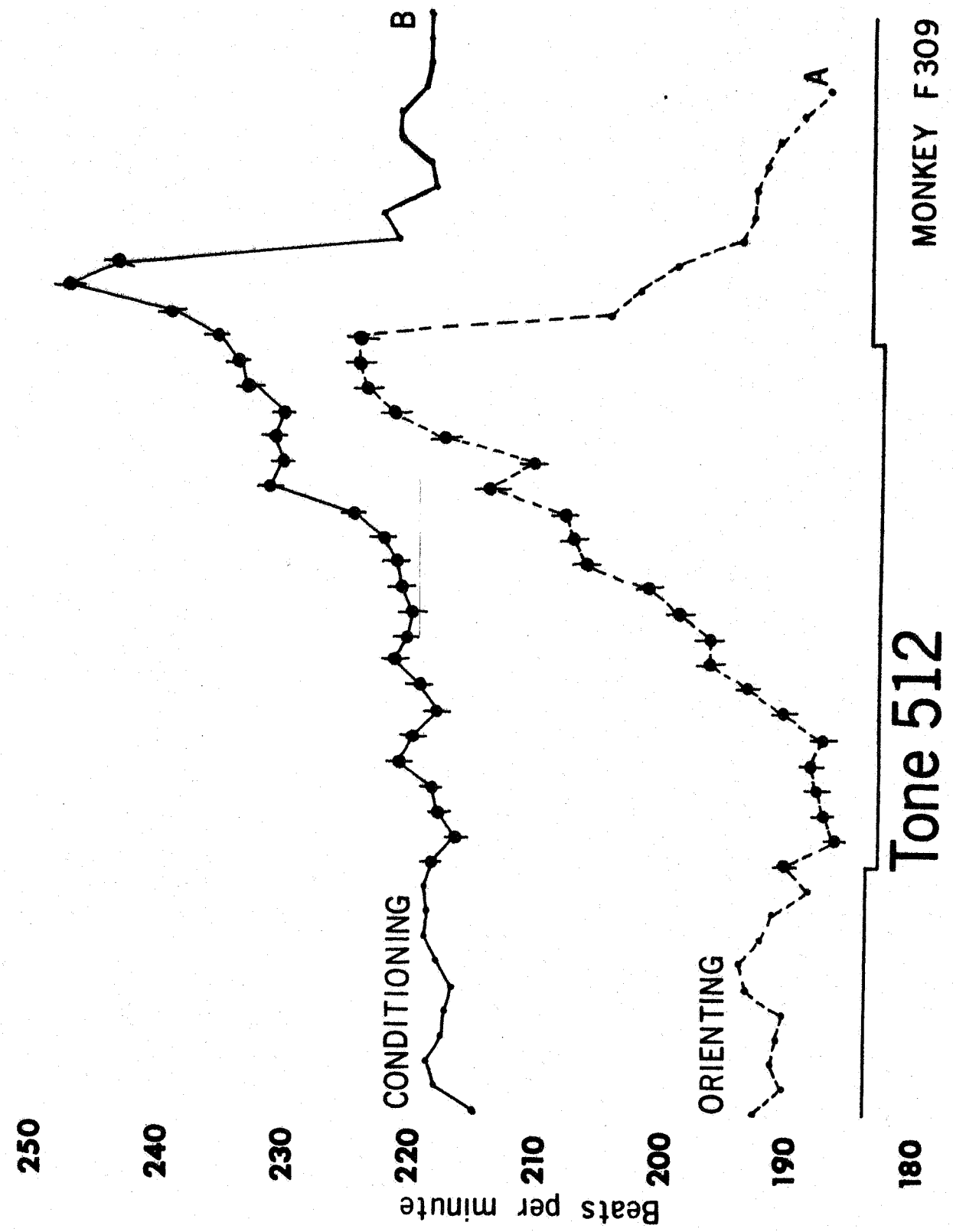
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Fig.4

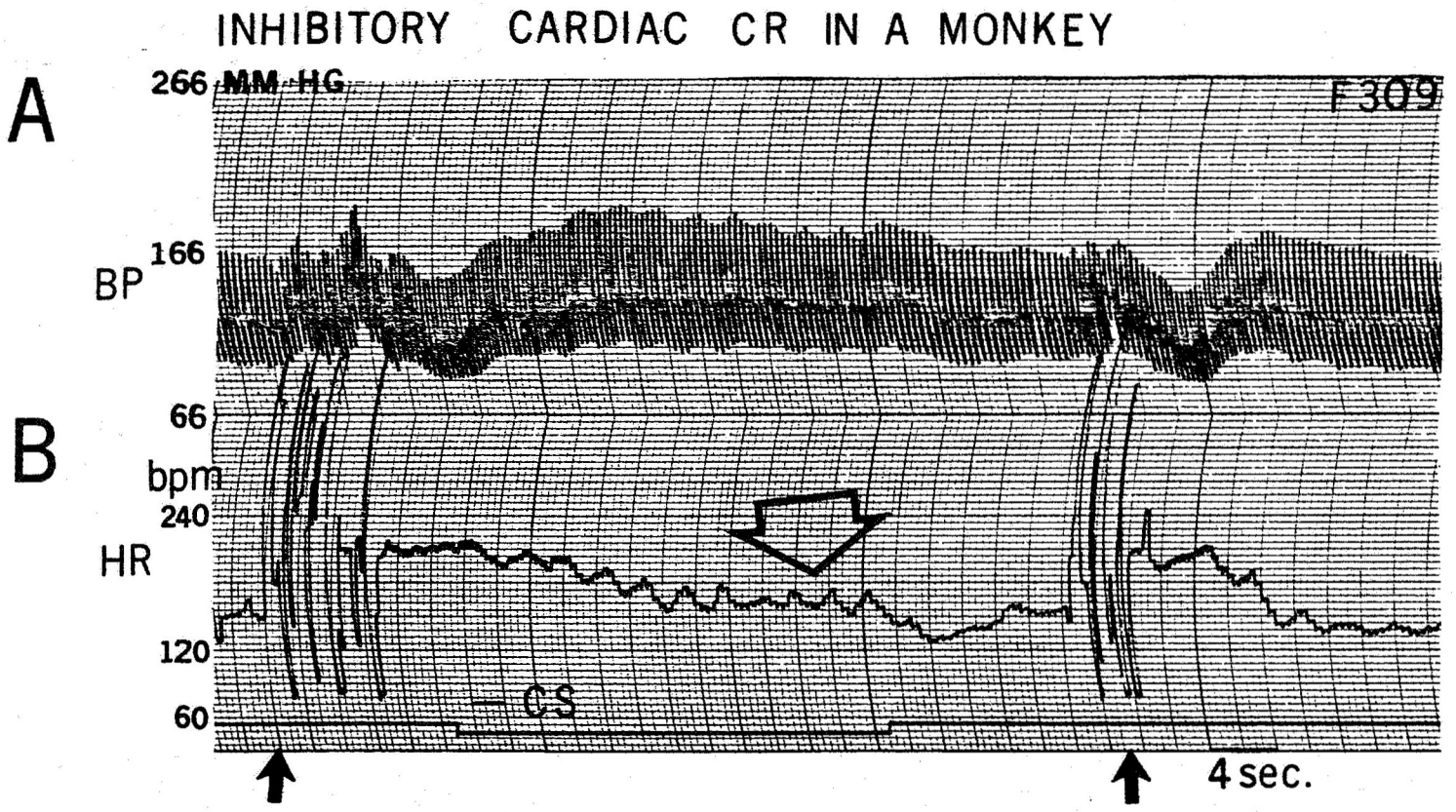

Figure 5: Tracing A illustrates intra-arterial blood pressure from abdominal aorta. Tracing B illustrates the heart rate beat-to-beat integrated by a Gilford cardiotachometer. Solid black arrows illustrate movement artifacts. Open arrow on top of the HR tracing indicates HR deceleration during -CS. Note that after the -CS is off there is a further drop in HR.

SUCCESSIVE-BEAT ANALYSIS OF CARDIOVASCULAR ORIENTING AND CONDITIONAL RESPONSES.

Cardiovascular orienting and conditioning in dogs have been studied by Gantt and co-workers for a number of years (Gantt & Hoffman, 1940; Gantt, 1944). Average increases in heart rate (HR) of 10 to 40 beats per minute (bpm) occur to an orienting stimulus (OS) such as a tone, viz. the orienting reflex (OR); the average increases diminish with repeated presentation of the stimulus (Robinson & Gantt, 1947). In conditioning experiments where the tone is reinforced with an electric shock to a leg as unconditional stimulus (US), the tone becomes a conditional stimulus (CS) which will elicit flexion of the leg and cardiovascular changes (Dykman & Gantt, 1960; Newton, 1963; Perez-Cruet & Gaertner, 1966).

The usual method for evaluating changes in heart rate and blood pressure (BP) during orienting or conditioning has been a comparison of the averages of counts for 5- to 6-second period of HR with systolic and diastolic pressures before, during, and after an OS or CS. This type of analysis gives no indication of beat-by-beat changes in cardiovascular parameters, which are important in the study of these reflexes.

We have developed a technique for averaging HRs and BPs on successive beats, over a large number of trials, before, during, and after an orienting or conditional stimulus.

Our method for measuring HR and BP resembles that used by Dykman and Gantt (1956) and by Teitelbaum and Gantt (1955) for measuring HR, except that ours does not involve a second-by-second analysis of HR. Zeaman, Deane, and Wegner (1954) have successfully used a method similar to that of Dykman, Teitelbaum and Gantt in evaluating HR in humans during conditioning. We have applied the same method to the analysis of BP. Before our study, the method had not been employed for evaluation of blood pressure in conditioning studies.

Method of Evaluating Heart Rate and Blood Pressure

In this paper we report HR changes in nine dogs during orienting and conditioning, using the successive-beat method; in six of these dogs BP changes were also evaluated.

EKG (normally a chest or standard limb lead) and intra-arterial BP from the abdominal aorta are recorded on an Offner-Beckman Type R polygraph. Respiration is monitored with a circum-thoracic strain-gage belt.

An on-line digital print-out of the heart period (cardiac-cycle duration) between each two successive R-waves, viz. the R-R interval (RRI), is obtained through a 5512A Hewlett-Packard eletronic counter. Heart period is punched manually on an IBM card printing machine.

Figure 1 shows the method of averaging successive beats before, during, and after a tone reinforced with a shock to the foreleg coming immediately after the tone. In these particular tracings the tone onset and offset and shock onset are synchronized with an R-wave of the EKG. During analysis all heart periods for several trials for the last R-R interval prior to stimulus onset (RRI-1) are converted by a computer technique to HR. They are then averaged and the standard deviation is computed. Working backwards in time, the same procedures are carried out for RRI-2 (next-to-last RRI before stimulus onset), RRI-3, and so on back to RRI-5 or RRI-10. Starting again at tone onset, the

average HRs and their standard deviations are computed for R-R interval +1, RRI +2, and up to RRI +8 or +10 during the tone. At tone offset averaging is begun again, working forward for 5 to 10 R-R intervals. The computed curve at the bottom of Figure 1 illustrates the beat-by-beat HR averaged for the seven trials shown.

To determine BP, a polyvinyl catheter is inserted through the femoral artery into the abdominal aorta by a technique described elsewhere (Perez-Cruet, Plumlee & Newton, 1966). Catheters remain in place and BPs can be recorded daily for 1 to 12 months. The outside end of the catheter is connected to a P23De Statham pressure transducer, taped to the thorax at the level of the heart, the output of which is recorded on the polygraph. Measurement of BPs is as follows: a technician measures manually, using the daily calibration, the diastolic trough and systolic peak of each pressure pulse in the succession, for each cardiac cycle in a given trial. The diastolic trough and systolic peak corresponding to the second of the two beats whose heart period is in question are taken as the BP for that particular R-R interval. The values of absolute pressure in mm Hg are then punched onto IBM cards for averaging across trials to give a mean systolic pressure, a mean diastolic pressure and their standard deviations for each beat of the succession, corresponding to the HRs.

Procedure Used to Train Dogs

Nine dogs were trained to stand quietly in a soundproof room. EKGs, respiration and movements of the left foreleg were continuously monitored. Dogs were restrained by means of a leash around the neck and a strap under the abdomen tied loosely to an overhead bar. In three dogs, BP catheters were maintained throughout the course of the experiment (2 to 3 months), in three other dogs direct BPs were recorded for only part of the experiment. No BP recordings were made for the remaining three dogs in the group.

Orienting Phase: Training consisted of presentation of tones which were later to become the CSs. Tones of 256 cps and 512 cps (low intensity, about equivalent to that of a loud whisper) were presented alternately at 2-minute intervals, usually ten times a day. For six of the dogs, each tone was sounded for 6 to 7 seconds at a time. For the other three dogs, the tone duration was the length of either R-R intervals, with triggering of tone-onset and tone-offset by an R-wave of the EKG. We have not found, in the dogs reported in this study, and definite differences in the effects on cardiovascular conditioning of these two slightly different methods of presenting the stimuli.

Conditioning Phase: After the orienting phase of 50 to 100 trials of each tone over a period of five to ten days regular reinforcement with shock was instituted, with all tones of one-frequency (256 cps for six dogs, 512 cps tones for three dogs) followed immediately by a 0.5- to 1.0-second AC shock (0.5 to 5.0 milliamperes) to the left foreleg. The dogs could never avoid the shock. Tones of the other frequency were never reinforced. Tone duration, intertrial interval, and order of presentation remained, for each dog, exactly the same as during the orienting phase. There were 25 to 150 trials of reinforced tones (CS+) and the same number of unreinforced tones (CS-) per dog (several daily sessions).

Extinction and Reconditioning Phases: In the extinction phase, the reinforcement was omitted and the CSs were presented alone for 30 to 100 trials for 3 to 10 days. During reconditioning, reinforcement to the foreleg was

resumed following all the tones of the same frequency used in the conditioning phase. Only two dogs were subjected to extinction and reconditioning.

Results

The results showed significant beat-by-beat changes in heart rate and blood pressure during orienting and conditioning.

Figure 2 illustrates beat-by-beat HR changes during the orienting and conditioning phases in dogs Night and Aggie. The analysis of the orienting phase included a total of 75 trials for Night and 60 trials for Aggie. The conditioning phase included a total of 153 trials for Night and 111 trials for Aggie. The orienting and conditioning trials were divided into series of 25 to 40 trials to determine consistency of the cardiovascular changes produced by the auditory signals, which produced either a motor OR in the orienting phase or a motor CR in the conditioning phase. The motor OR consisted of moving, turning the head, and looking around. The motor CR consisted of defensive withdrawal and lifting of the foreleg to which the US was applied. During the orienting and conditioning phases there was a sudden initial HR-decrease at the onset of the auditory stimuli. The initial deceleration of HR varied between 5 and 25 bpm below pre-stimulus HR and occurred on the first cardiac cycle after the onset of the tones. During orienting trials, immediately after this initial deceleration of HR the rate began to increase slightly in Night and more significantly in Aggie. In Aggie, the acceleration of HR during OR was more accentuated to the tone of 512 cps than to the tone of 256 cps. During the conditioning phase, the HR-CR consisted of an acceleration in HR of about 30 beats above pre-stimulus levels. In Night (upper curves), the HR-CR was different from the HR-OR in the degree to which acceleration occurred as a CR and also in terms of differences in heart rate responses to tones of CR and also in terms of differences in heart rate responses to tones of 256 and of 512 cps, as shown in Figure 2. In Aggie (lower curves), the results showed some similarities between the HR-OR and the HR-CR, but the latter showed a greater acceleration during the CS+ (especially conditioning series 4). The results shown in Figure 2 illustrate differences in the response patterns of the HR-OR and the HR-CR. However, it is evident that in these two dogs the initial deceleratory component at the onset of the tone persisted throughout orienting and conditioning. Another interesting finding was that, while no clear differentiation of the sudden initial deceleratory response was observed during orienting, after conditioning there was a tendency for it to become more accentuated during reinforced CS.

Intra-arterial systolic and diastolic BP and HR were measured concurrently during conditioning in four dogs (Fig. 3A, 3B). BP, in the four dogs, followed the HR except that the BP-CRs were not as prominent as the HR-CRs. Helen and Foxy showed no differentiation in HR between the two tones during orienting, whereas during conditioning the HR-CR was differentiated. Foxy had a HR-CR about 60 beats above pre-stimulus levels, much more prominent than HR-OR. The US produced an unconditional acceleration in heart rate (HR-UR) (Fig. 2,3A,3B, small arrows) with the maximum value higher than the peak value of the HR-CR. On the other hand, the BP unconditional reflexes (BP-URs) were confounded because, in spite of consistent accelerative HR-URs, Helen and Foxy showed decreases in systolic and diastolic BP-URs; another dog (Mike) showed an increase in systolic and diastolic BP as a UR; and the fourth dog (Fancy) showed an increase in diastolic but not systolic BP changes as a BP-UR.

Figures 4A & 4B show the results of extinction and reconditioning procedures in Eloise and Jeff. In Eloise for more than 30 trials during the first four experimental sessions while the reinforcement was entirely omitted, the HR increase during the positive CS continued practically unchanged, with good differentiation maintained. Eventually, after ten sessions (the last four of which are shown under "later extinction" in Figure 4A), the HR increase diminished markedly, though there was still a small HR increase to the CS+, with differentiation. Reconditioning, during which reinforcement was resumed, reestablished the HR increase at the pre-extinction level. The other dog, Jeff, which received a shorter series of extinction trials, showed extinction of the HR-CR to the CS+; but the sudden HR-decrease and BP-decrease to the CS+ continued to occur just as during the conditioning procedure, whereas the CS- continued to produce a less prominent sudden HR-decrease or BP-decrease. Reconditioning caused reestablishment of the subsequent HR and BP CRs, with differentiation.

As shown in Figure 5, one dog (Queenie), after development of CRs, underwent a procedure which we found useful in attempting to delineate the role played by muscular and respiratory movements in cardiovascular conditioning. In order to rule out the possibility that the HR-CR and BP-CR were secondary to muscular movements, we administered d-tubocurarine in the dose of 0.5 mg/kg and, on a later occasion, succinylcholine, 10 mg/kg intravenously. The dog was paralyzed by the drugs and was ventilated artificially for 30 to 40 minutes. As Figure 5 shows, BP-CRs were only slightly affected by the drugs, whereas muscular movements of all types, including respiratory movements, were abolished.

Analysis of the data from all the dogs (see Figs. 2 through 5) indicates that four of them exhibited the prominent sudden initial HR-decrease to tones during orienting training. In two of these (Aggie, Jeff), during conditioning the initial deceleration of HR occurred more prominently than during orienting. In addition there was greater deceleration in response to the CS+ than to the CS-, which is evidence of differentiation between tones with respect to the initial HR-drop. Night continued to show the prominent deceleration, but without consistent differentiation between tones with respect to the initial HR-drop. Night continued to show the prominent deceleration, but without consistent differentiation between the tones. The last of the four dogs (Helen) showed no initial HR-decrease after conditioning. All four dogs which, prior to conditioning, had not shown the initial deceleration, after conditioning showed it to some extent. Queenie showed the marked deceleration characteristic of some of the previous dogs (20 to 30 bpm HR-drop). Foxy, Mike, and Eloise showed only small initial HR-drops of 5 to 10 bpm. The ninth dog, on which there were no data prior to conditioning, showed 20 bpm initial HR-deceleration during conditioning. HR-acceleration, beginning on the second to fourth cardiac cycle after onset of the CS+ occurred in all nine dogs. Five dogs (Night, Aggie, Helen, Mike, Jeff) showed similar HR-acceleration phenomena: the HR reached a maximum on the third to seventh cardiac cycle after onset of the CS+, thereafter remaining on a plateau 30 to 40 bpm above baseline or returning to baseline while the CS+ continued. Fancy showed a similar plateau effect, with only a 15 bpm HR-increase above the baseline until CS termination. In contrast to the other six dogs, Foxy, Eloise, and Queenie showed a steadily accelerating HR throughout the duration of the CS+, maximum HR by the end of the CS+ being 40 to 130 bpm above the pre-CS baseline.

Differentiation between CS+ and CS- was excellent in Night, Helen, Foxy, and Eloise (large HR-increase to reinforced tones, little change to unreinforced tones); good in Aggie and Jeff (large HR-increase to reinforced tones, smaller change to unreinforced tones); and poor in Mike, Fancy, and Queenie (little or no difference in effect of reinforced vs. unreinforced tones).

During conditioning both systolic and diastolic BP followed HR fairly well with respect to the early part of the CS+. That is, there were initial drops in systolic and diastolic pressures simultaneously with the initial HR-deceleration. BP increased considerably during the subsequent HR-acceleration. However, during the latter part of the acceleratory phase, Foxy's BP decreased while HR continued to rise, and Fancy's BP continued to increase while HR dropped. Differentiation between tones showed more prominently in BP than in HR in both Fancy and Queenie. BP was a better indicator of conditioning than HR in Helen and Jeff, whose upper absolute HR-CR levels were little different from those during HR-OR. In these two dogs, the BP-CR levels were 15 to 20 mm Hg higher than the BP-OR.

Statistical Tests: Standard deviations for most of the dogs tended to be rather large (10-40), especially for HR, due to the prominent sinus arrhythmia. Despite this large variability, t-tests usually showed statistically significant changes during conditioning. In the orienting sessions none of the eight dogs showed significant differences between the average maximum HR reached during tones of 256 cps and the average maximum HR during tones of 512 cps, though the absolute HR acceleration from baseline was significant in five dogs (Aggie, Helen, Foxy, Mike, Jeff). In contrast, during conditioning the differences between average maximum HR to CS+ and maximum HR to CS- were significant in five dogs (Night, $p < .001$ in series 1 and 4; $p < .01$ in series 2 and 3; Helen, $p < 01$; Foxy, $p < .001$; Eloise, $p < .001$; Jeff, $p < .001$). Aggie, in conditioning series 4, also showed a significant difference ($p < .01$) between maximum HR to CS+ and maximum HR to CS-. During conditioning the absolute HR acceleration from baseline to CS+ or to both tones was significant in eight of the nine dogs (it was not significant in Fancy).

Discussion

The use of this method allows detailed analysis of cardiovascular reflexes during orienting and conditioning. In many dogs there is a biphasic cardiovascular reflex, that is, initially HR and BP tend to decrease suddenly for one or two cardiac cycles, sometimes very markedly, and after a few beats there is an increase in HR and BP above a pre-stimulus baseline. This biphasic pattern is not evident in HR obtained by counting the number of beats in the whole period of the CS, though it can sometimes be seen in a second-by-second count of HR. Zeaman and Smith (1965) have shown that the HR change in humans during CS for shock is also biphasic, though the phases are reversed from those in the dog; that is, there is an initial acceleration and a subsequent deceleration.

This method gives us a means of determining the latent period of the cardiovascular changes during orienting and conditioning. From the curves we determine the duration of time from the middle of the last R-R interval before CS onset until the middle of the R-R interval representing first significant HR increases above the baseline, which would appear to be the best approximation of the latent period of a HR increase. By this method, the latent period of the HR increase varied from 1.3 to 1.6 seconds in our nine dogs during orienting and conditioning. A more certain, but less sensitive, measure of the latent period is 2 to 4 cardiac cycles in these same dogs.

The fact that decreases in heart rate and blood pressure occurred immediately after CS onset in several of the dogs during the orienting training suggests that these responses were components of the OR. How are we then to interpret the finding that two of the dogs during conditioning showed this response to a pronounced degree to the positive tones and very little to the negative tones? These data suggest that the initial HR decrease can participate in the cardiovascular CR and even in differentiation, though the differentiation of it seems to be more difficult than the differentiation of the subsequent HR and BP increase, whereas only two dogs showed consistent differentiation of the initial decrease. The initial HR decrease probably represents an OR, which in some dogs tends to extinguish during the CS- while becoming more pronounced during the CS+. This would mean that some OR components participate in conditioning and differentiation, as postulated by Sokolov (1960). In one of our dogs (Helen), the HR increase occurring on the second to fifth beats during the CS+ was similar in form and magnitude to the HR increase during orienting, whereas the HR increase during the CS- was much less than that during orienting, indicating that differentiation had been established. Thus, it seems that orienting plays a part in conditioning, as logical and experimental considerations would lead us to expect. However, it is difficult to ascribe only to orienting the large HR and BP changes seen in several of our dogs.

Our findings of retention of the massive HR increase to the CS+ in one dog (Eloise) and retention of the differentiation between CS+ and CS- with respect to the initial bradycardia in another dog (Jeff) during the extinction phase support Gantt's (1960) observation that cardiac CR's extinguish with difficulty. Extinction of the HR increase eventually did occur in Eloise, but only after nine experimental sessions, or more than 80 unreinforced trials of the CS+.

In our experiments respiration was constantly monitored in order to determine to what extent respiratory changes influenced the biphasic HR-CR. There was no definite evidence that the HR-CR was an artifact of respiration. The relationships between the HR-CR and respiration were not simple. Wood and Obrist (1964) have confirmed that there is a definite HR deceleration in humans as a defensive HR-CR, which seems to be independent of respiration; on the other hand, these authors have shown that the initial components of the biphasic reflex, especially the initial accelerative component, may be secondary to a respiratory change. Smith (1963), in another series of experiments, obtained an accelerative response during breath holding, which indicates that the defensive HR-CR in humans is modified by breath holding. The idea that respiratory changes influence the cardiovascular system by means of reflexes mediated through pulmonary afferents is not a novel one. Freedman (1951) has shown that respiratory changes occur during conditioning. Undoubtedly cardiovascular CRs are modified by respiratory changes. In fact, respiratory maneuvers can be used as a US for establishing a cardiac CR (Perez-Cruet, 1962). Nevertheless, we hypothesize that both cardiovascular and respiratory CRs stem from some common excitatory influence in the central nervous system, which initiates essentially simultaneous outflow of impulses into both of these organ systems. The evidence for this hypothesis derives from cardiovascular changes in animals paralyzed by curare (Newton and Gantt, 1960) and instances of conditioning where there is dissociation between cardiovascular and respiratory changes (Malmo, 1963; Perez-Cruet and Gantt, 1961). Perez-Cruet, Newton and Gantt's (1965) observations of sinus arrhythmia dissociated from respiration during steady panting also indicate that cardiovascular changes can occur independently of respiration. Furthermore, there is evidence that the cardiovascular CRs are established prior to motor and respiratory CRs (Pinto, Newton and Gantt, 1957; Gantt, 1960).

Our experiments with d-tubocurarine and succinylcholine indicate that neither respiratory nor proprioceptive reflexes account for the BP-CR. The dog was totally paralyzed and was adequately ventrilated by positive pressure at a regular rate of 18 cycles/min. The reduction of the HR changes during the CS+ might be interpreted as indicating that the HR-CR is secondary to movement and/or respiratory changes, whereas the BP-CR is primarily due to a purely central excitatory effect. Another more likely interpretation is that the central neural control of HR, through the cardiac nerves, is more markedly affected by these drugs than is the neural control of BP, thereby reducing the magnitude of HR responses to centrally mediated stimuli. Evidence for the latter interpretation is noted in the remarkable overall HR decrease due to the d-tubocurarine and the succinylcholine, and in the fact that the US itself caused very little HR change under the drugs. Perez-Cruet, McIntyre, and Pliskoff (1965) found that curarization markedly reduced the HR response to hypothalamic self-stimulation, leaving unaffected the BP response to such stimulation. We have also observed, in experiments on other dogs totally paralyzed with d-tubocurarine, the sudden HR and BP decrease which commonly occurs at the onset of the CS. Black, Carlson and Solomon (1962) have also reported both HR-increase and HR-decrease during the CS in dogs paralyzed with curare.

For many years, we have analyzed HR-CRs by averaging the number of beats per 5- or 6-second intervals across many trials. Therefore the curves which we have previously obtained represent an overall average. This, perhaps, may be considered a disadvantage when one is interested in measuring trial-by-trial changes in HR. Trial-by-trial analysis of orienting and conditioning cardiac data usually reveals differences between these two responses, as noted in the review by Graham (1966) and in unpublished studies by Lynch (1966). These investigators point out that the initial trials of an orienting stimulus usually produce HR deceleration whereas the conditioning is usually acceleratory or biphasic. Since our data represent averages of many trials, the acceleration usually seen as the HR-OR may be due to adding in the acceleratory change noted by Graham as characteristic of later orienting trials.

The overall HR-decrease (no subsequent increase) seen in some dogs during the CS+, as noted in a footnote above, appears to be a HR-CR, since differentiation occurs in some of the dogs (little or no HR-decrease to CS-). Whether this is conditioning of a cardiac-orienting response is a question to be resolved by future experiments.

Our data show that each dog has its own cardiovascular pattern of responding to the CS, viz., cardiovascular CR, which is fairly consistent from session to session and in some dogs tends to transfer in a slightly modified form or only in magnitude of change from the orienting training to the conditioning situation. In addition to these sometimes distinct individual patterns of cardiovascular responding, there are general patterns of cardiovascular orienting and conditioning characteristic of the species. The data reported in this paper show both the general patterns characteristic of the dog and some of the differing patterns among different dogs.

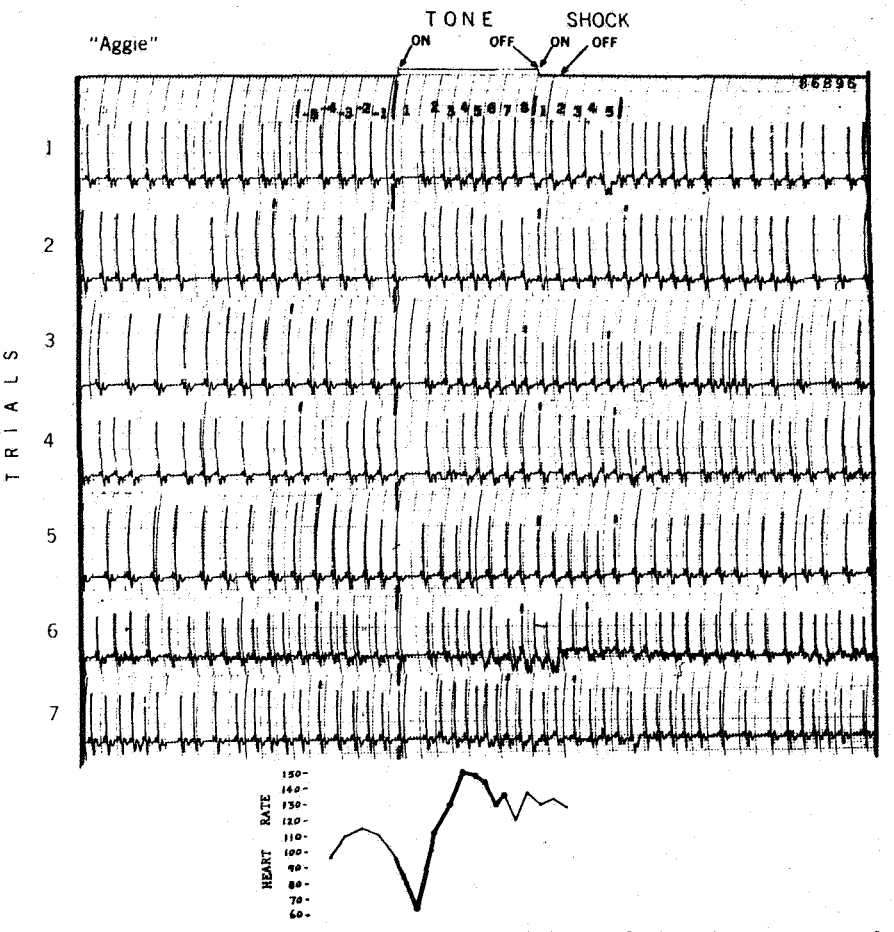

FIG. 1. Method of averaging successive R-R intervals (RRI) across several trials of a CS. Onset and offset of the tone and onset of the shock US are synchronized with an R-wave of the EKG. In these tracings the tone-duration was 8 RRI. During analysis the last RRIs prior to tone onset (RRI −1) for all 7 trials were converted by a computer to HR, then averaged. Working backwards, the same procedures were performed for RRI −2 (next to last RRI before tone onset), RRI −3, RRI −4, RRI −5. Starting again at tone onset, the average HR's were computed for RRI +1, RRI +2, up to RRI +8 during the CS. After tone offset averaging continued for 5 beats, as above. The curve at bottom shows the beat-by-beat HR averaged for the 7 trials, the tone-period being represented by the heavier black line.

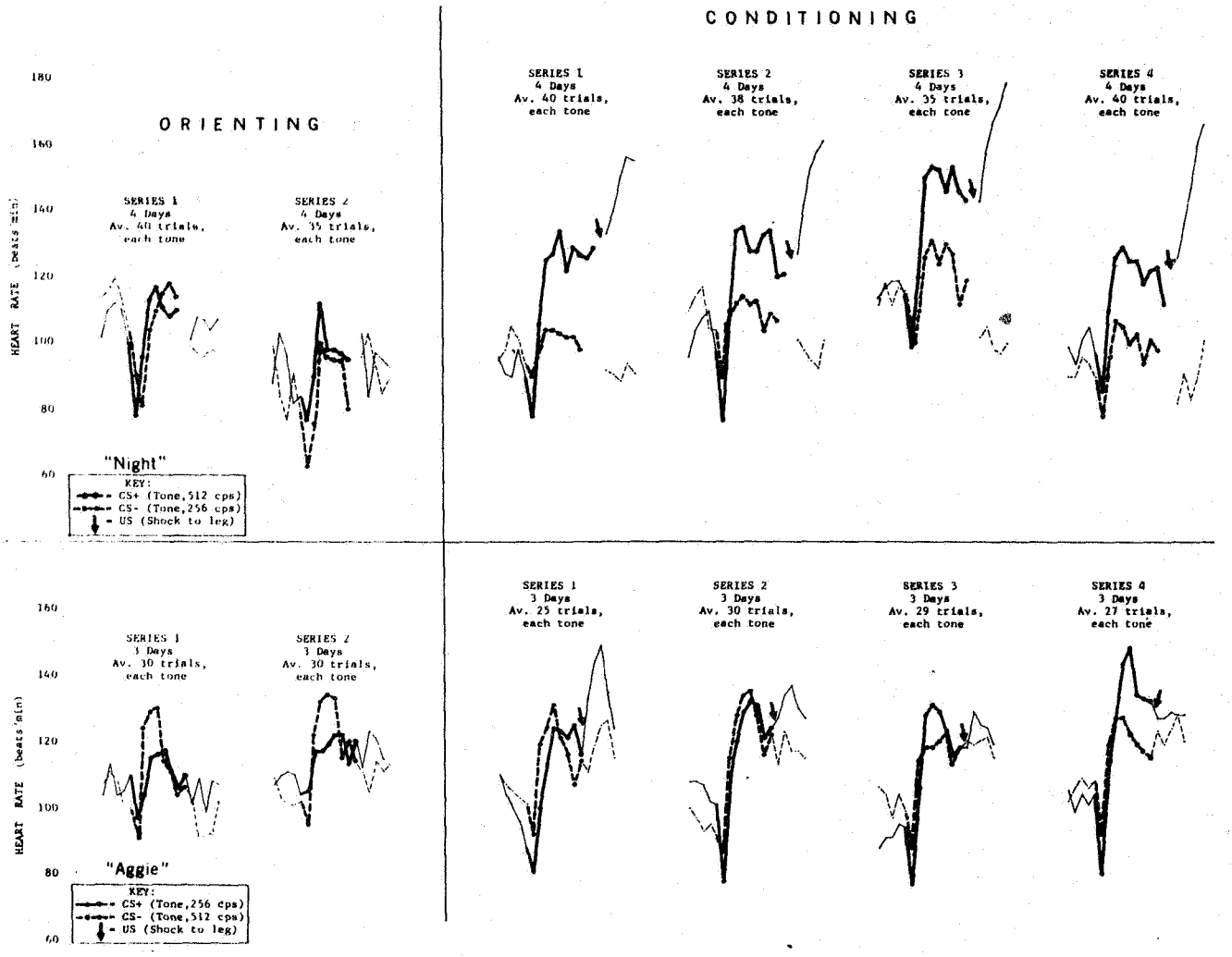

FIG. 2. Average beat-by-beat HR changes during several series of orienting and conditioning experiments in 2 dogs. Heavy solid lines represent HR during tones (CS+) which, during conditioning series, are followed by shock to foreleg (arrows). Heavy dotted lines represent HR during tones of another pitch (CS−), which are never reinforced.

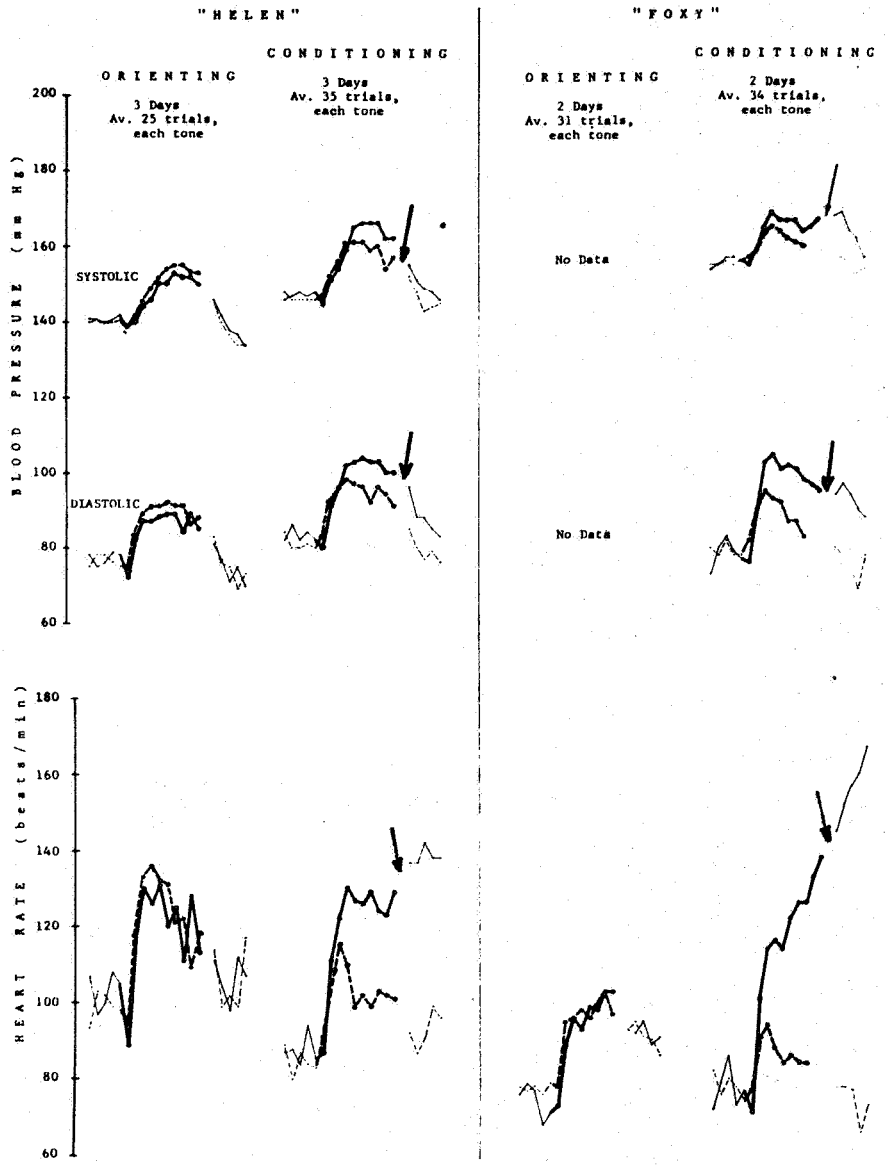

FIG. 3A. Average beat-by-beat BP (systolic and diastolic) and HR changes in 2 dogs during orienting and conditioning. BP was not recorded in Foxy during orienting. Explanation of symbols is the same as in Fig. 2.

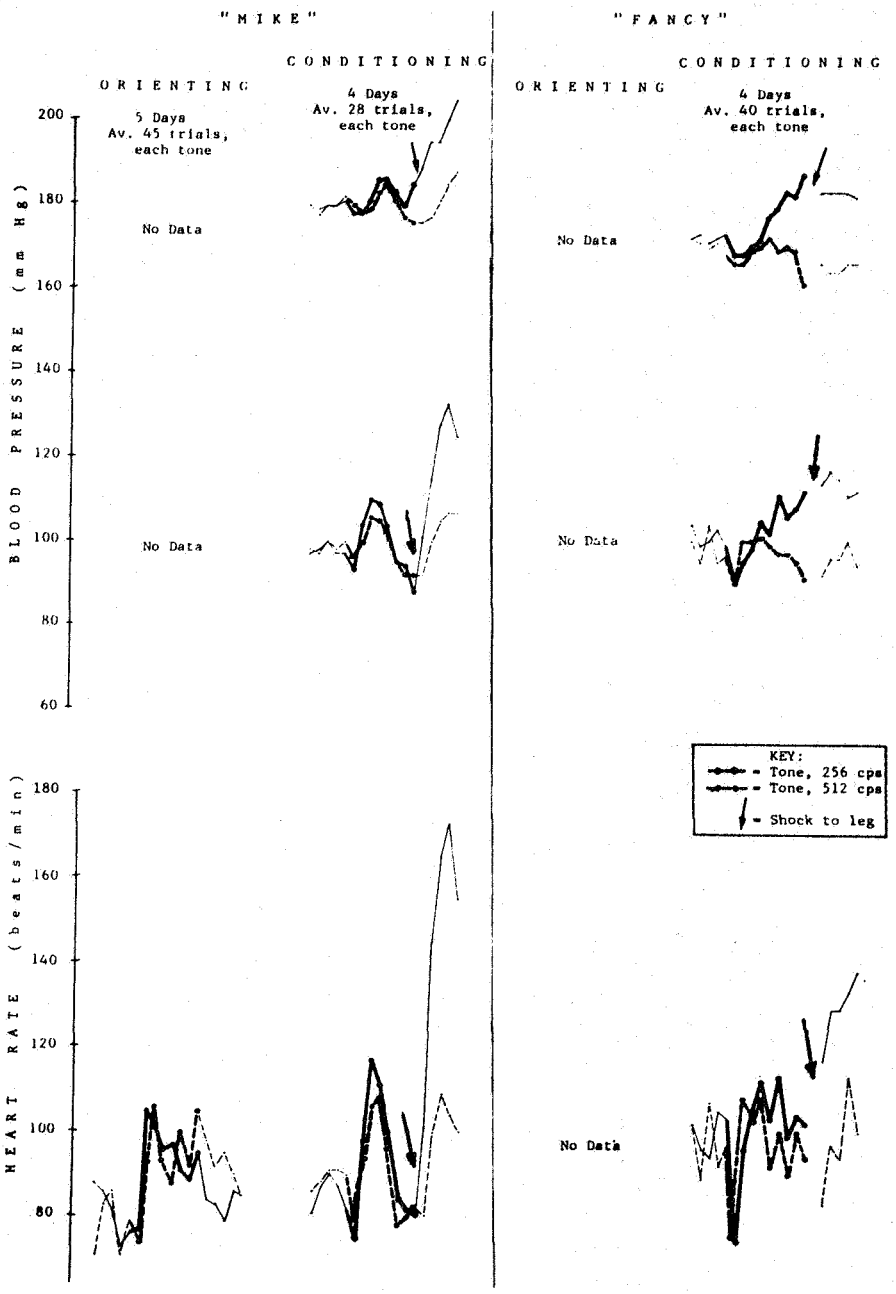

FIG. 3B. Average beat-by-beat BP (systolic and diastolic) and HR changes in 2 dogs during conditioning. HR was measured in Mike during orienting. Explanation of symbols is the same as in Fig. 2.

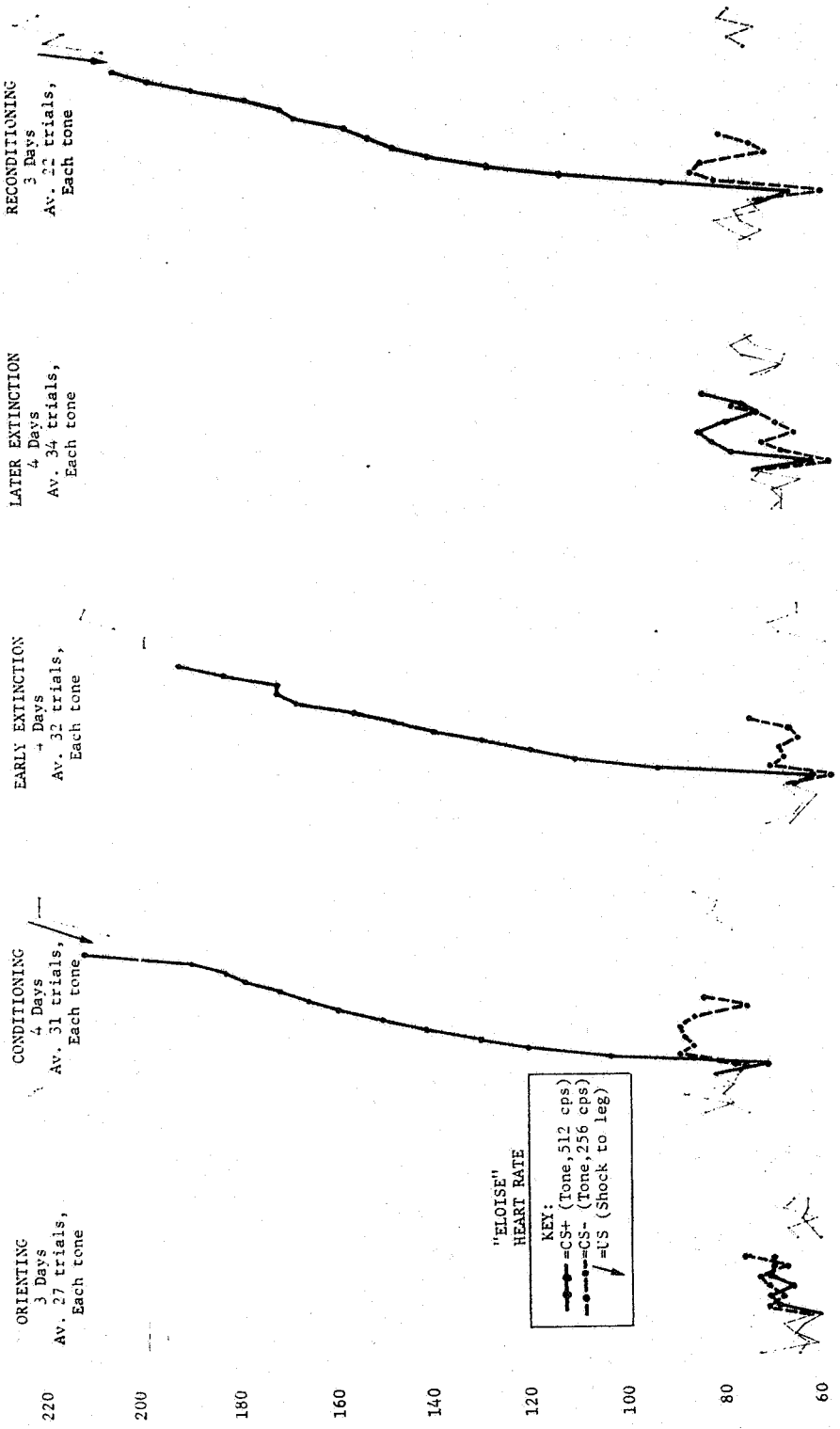

FIG. 4A. Heart rate during orienting, conditioning, extinction, and reconditioning in a dog. See Fig. 2 for explanation of symbols and text for explanation of procedures.

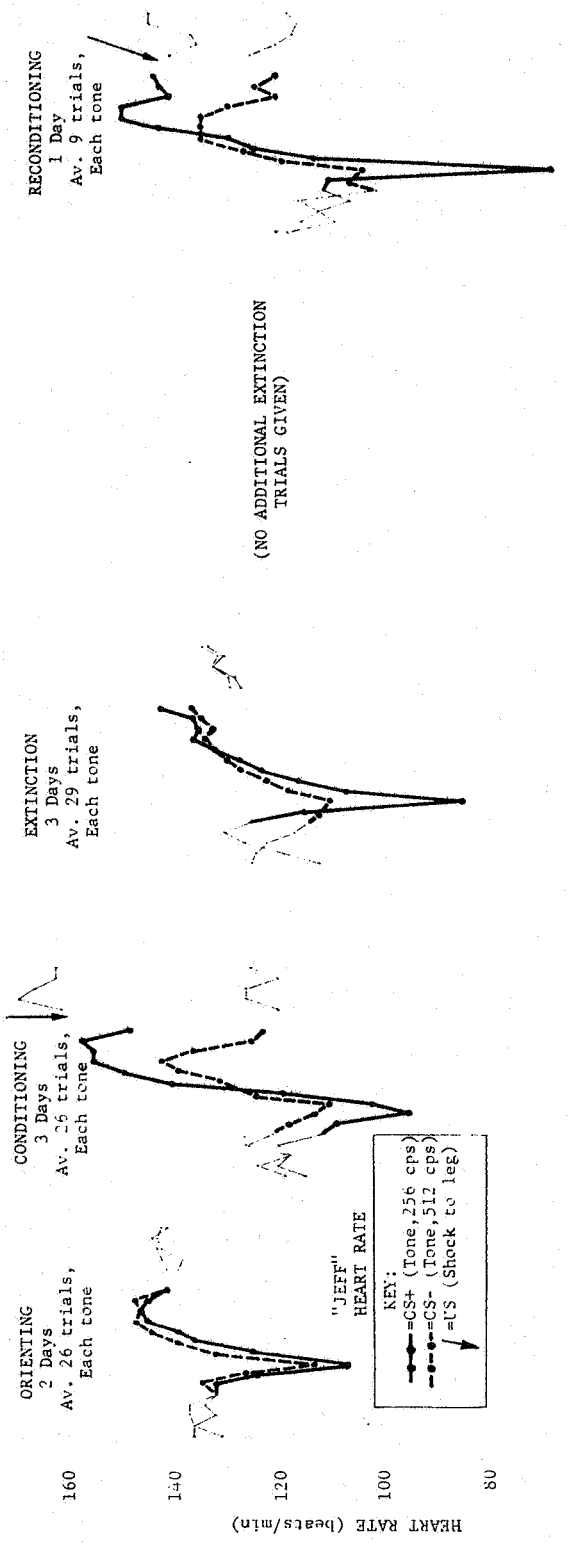

FIG. 4B. Heart rate and blood pressure during orienting, conditioning, extinction, and reconditioning in a dog.
See Fig. 2 for explanation of symbols and text for explanation of procedures.

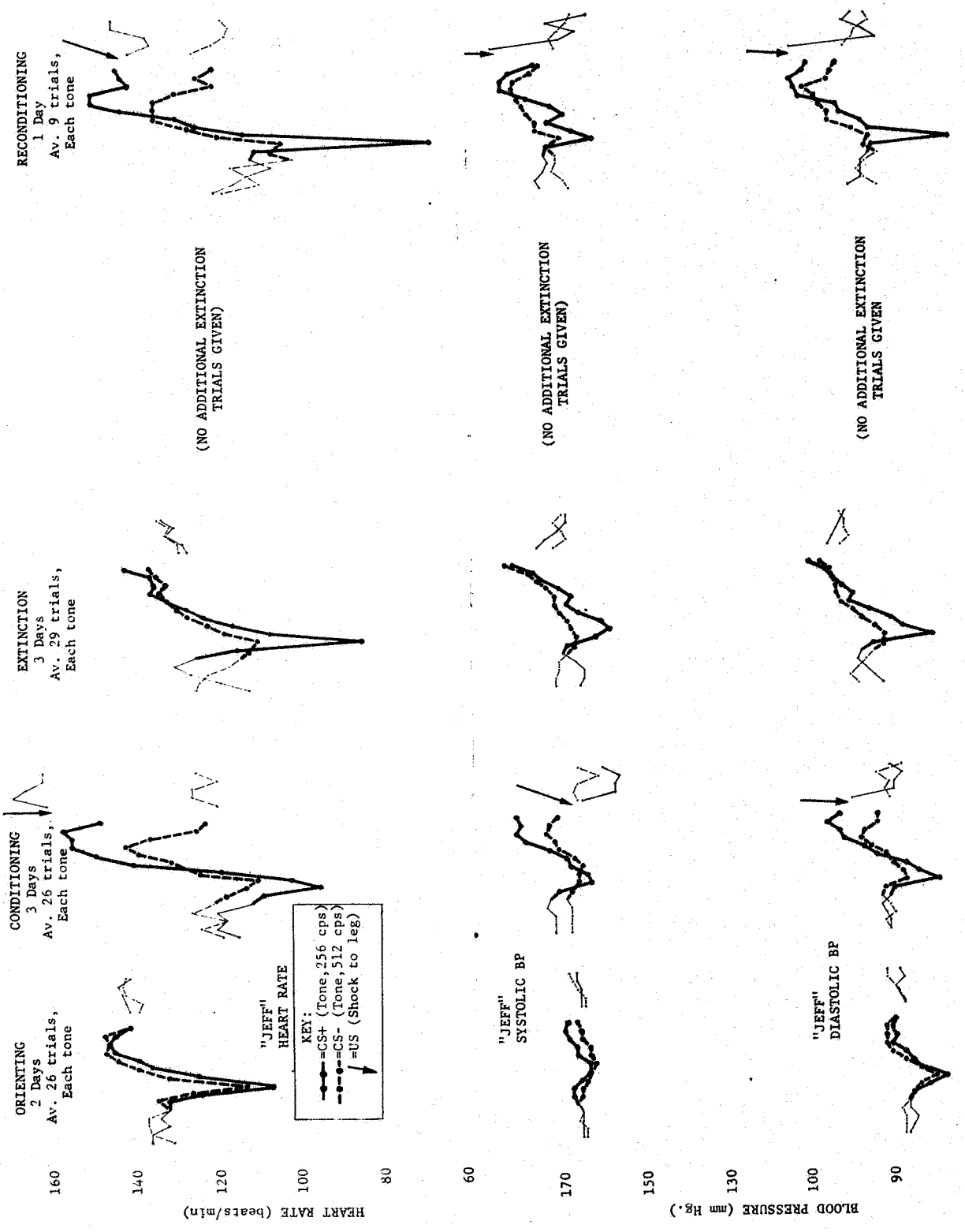

FIG. 4B. Heart rate and blood pressure during orienting, conditioning, extinction, and reconditioning in a dog.
See Fig. 2 for explanation of symbols and text for explanation of procedures.

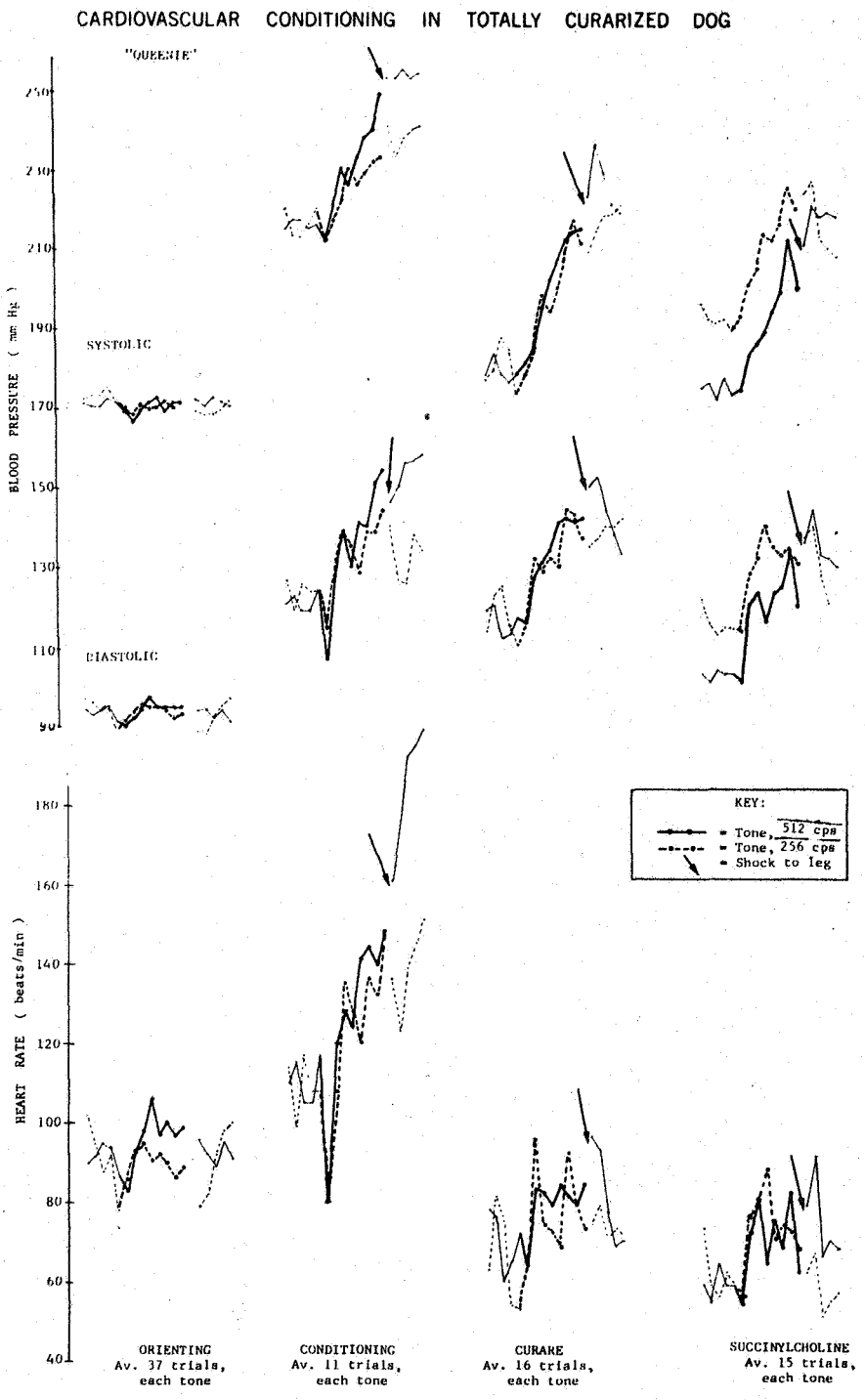

FIG. 5. Cardiovascular CRs in a dog temporarily paralyzed in separate experiments with d-tubocurarine and succinylcholine. The dog was artificially ventilated during each drug session. Explanation of symbols is the same as in Fig. 2.

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CERTAIN FEATURES OF CARDIODYNAMIC CHANGES DURING CARDIAC CONDITIONING IN DOGS.

The heart rate conditional reflex (HR-CR) in dogs usually consists of a progressive acceleration in heart rate (HR) during a conditional stimulus (CS) previously reinforced by aversive stimulation to a foreleg. The pattern of the HR-CR in some dogs can also be biphasic or strictly deceleratory.

In conditioning experiments the HR-CR is one of the many autonomic components accompanying the motor conditional reflexes. In some experiments, however, an independence between motor, respiratory and cardiovascular conditional reflexes has been observed. The independence between voluntary (motor) and involuntary (autonomic) conditional reflexes suggest that in certain instances the central nervous system can influence specific areas of the body without mass involvement of physiology systems.

The main purpose of this experiment is to determine changes in various physiological parameters within the cardiovascular system and to study the dynamics of these functions during classical heart rate conditioning. For this purpose techniques for measuring aortic blood flow (ABF), right ventricular pressure (RVP) and aortic blood pressure (BP) chronically in unanesthetized dogs were developed. This experiment is also designed to study the relations between the above cardiovascular functions in order to determine the vascular mechanisms through which the cardiac conditional reflexes can be mediated.

In six dogs electromagnetic blood flow probes have been implanted around the ascending aorta for measuring aortic blood flow; intra-arterial catheter have been inserted either through the common carotid artery and/or femoral arteries to measure ascending and abdominal aortic blood pressures with techniques described elsewhere by Perez-Cruet, et al. (1966); in three of the above dogs catheters were inserted through the wall of the right ventricle to measure systolic right ventricular pressures. The above intra and extra vascular placements are illustrated in Diagram 1.

The beat-to-beat analysis of an accelerative heart rate conditional reflex to painful stimulation has revealed consistently an increase in the systolic right ventricular pressure and a slight diminution in the amplitude of the peak aortic blood flow at the onset of the accelerative HR-CR as shown in Figures 1 and 2. Experiments are in progress to elucidate the dynamics involved in these cardiovascular changes associated with the HR-CR. It is premature to conclude that the heart rate acceleration as a HR-CR is mediated by the above changes in peak aortic blood flow or systolic right ventricular pressure; however, the data suggest that these cardiac functions are associated with the cardiac acceleration produced by the conditional nervous excitation of the heart.

Differential effects from other forms of nervous excitation on the heart such as the effect of a person entering the experimental room or painful stimulation of the skin with an electric shock were obtained. These stimulations produced consistently an increase in the amplitude of the peak aortic blood flow and an increase in the systolic right ventricular pressure as shown in Figures 3 and 4.

The thoracic and abdominal aortic blood pressure showed an increase from 160 to 190 mm Hg. during a reinforced conditional stimulus (+CS), but no change to another non-reinforced conditional stimulus (-CS). See Figure 5.

In summary, the cardiac conditional reflexes consisted of progressive acceleration in HR which varied from 10 to 60 beats above pre-stimulus HR levels. This progressive acceleration in HR was accompanied by an initial slight decrease in the peak aortic blood flow representing stroke volume and by an increase in the systolic right ventricular pressure. Thoracic and abdominal blood pressures were also increased during the +CS. The changes in peak aortic blood flow during cardiac conditioning differ from those seen during excitement due to the effect of person where the peak aortic blood flow was increased. The studies demonstrate that other cardiovascular functions such as peak aortic flow and right ventricular pressures also changes during heart rate conditioning.

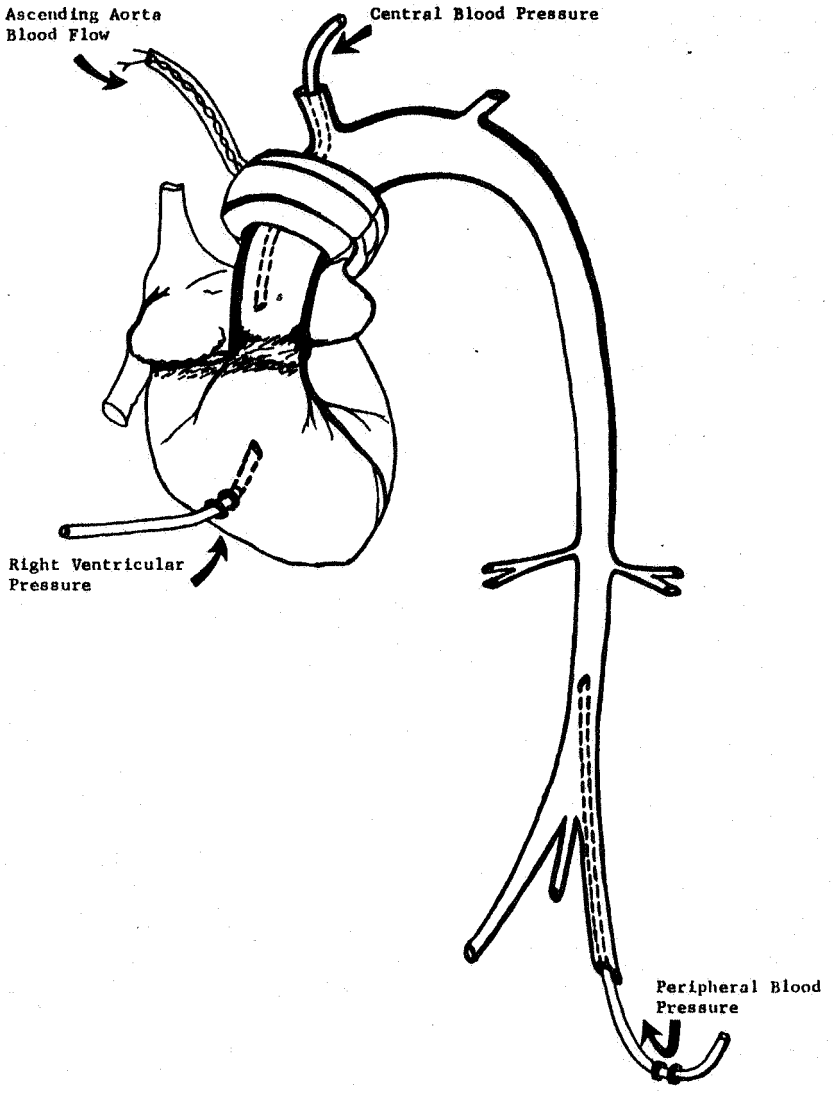

DIAGRAM 1: Diagram illustrates electromagnetic flow probe implanted around the ascending aorta for chronic measurement of aortic blood flow; implantation of intra-arterial catheter through brachio-cephalic artery into ascending aorta (Central blood pressure) and/or through femoral artery into abdominal aorta (Peripheral blood pressure); and intra-cardiac catheter inserted through the wall of the right ventricle to measure systolic right ventricular pressure. Chronic measurements have been taken for periods ranging from 10 days to 2½ months.

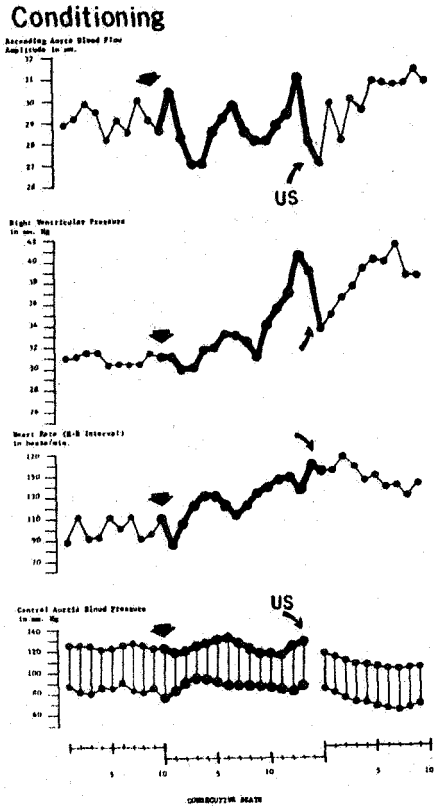

FIGURE 1: Changes in the amplitude of the peak aortic blood flow; systolic right ventricular pressure in mm Hg.; heart rate in beats per minute; R to R interval; and central aortic blood pressure (direct intra-arterial with the tip of the catheter in the ascending aorta as shown in Diagram 1). Note that there is a decrease in the peak aortic blood flow and an increase in right ventricular pressure during the CS as shown by the first heavy arrow. The data represent an average of five trials.

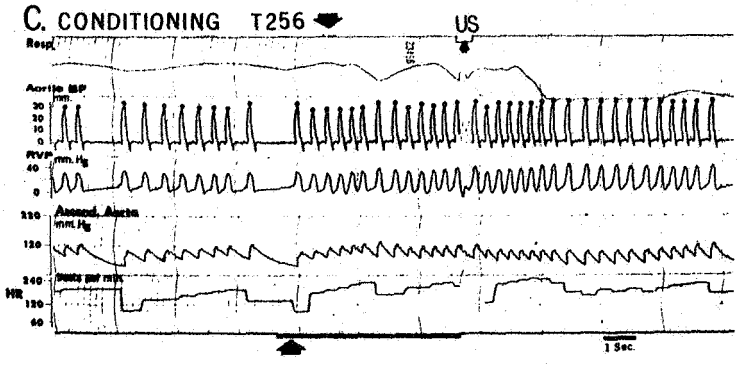

FIGURE 2: Changes in respiration, aortic blood flow, right ventricular pressure, ascending aortic blood pressure and beat-to-beat heart rate during a reinforced tone T256. US is the unconditional stimulus, namely a painful electric shock to the skin. Dots on top of the pulsatile blood flow curve were inserted by hand to aid the visual inspection of the changes in peak aortic blood flow. Note the initial diminution in the amplitude of the pulsatile blood flow at the beginning of T256 (see heavy arroe), but an increase in the right ventricular pressure, heart rate and respiratory rate during presentation of the conditional stimulus (T256).

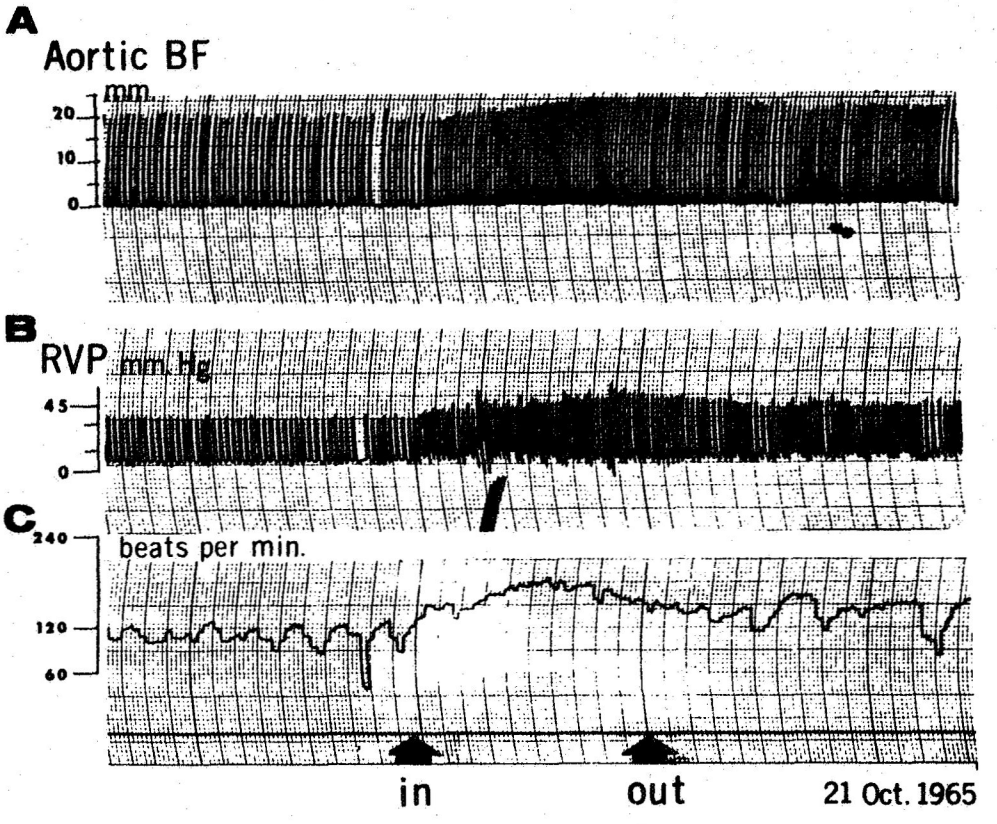

FIGURE 3: Tracing illustrating pulsatile ascending aortic blood flow, right ventricular blood pressure, and beat-by-beat heart rate from a Gilford cardiotachometer when a person enters the experimental room. Heavy arrows indicate person entering (in) or leaving (out) the experimental soundproof room. Note that the excitement produced by the person entering the room produces an increase in the amplitude of the aortic blood flow and simultaneously increases in right ventricular pressure and heart rate. In this tracing 1 mm change in the pulsatile blood flow represents approximately 90 cc/min. of blood flowing through the flow probe.

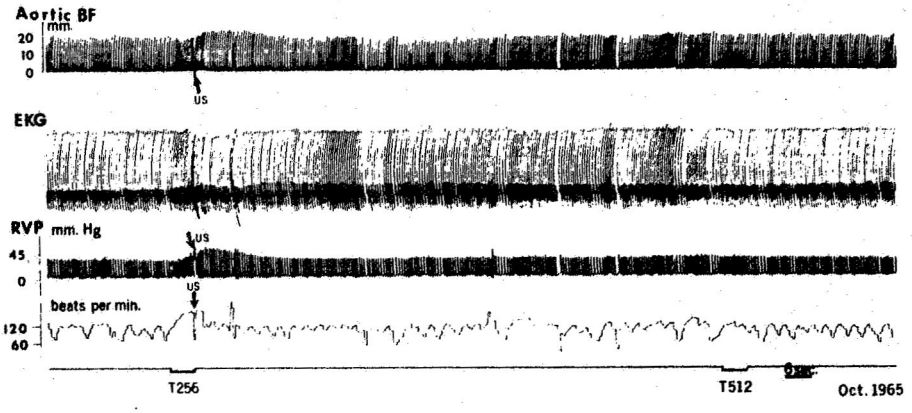

FIGURE 4: Tracing illustrates aortic blood flow, electrocardiogram, right ventricular pressure and heart rate before, during and after a reinforced conditional stimulus T256 and to a non-reinforced conditional stimulus T512. Changes in these cardiovascular parameters to a painful electric shock (US) to the skin are seen after arrows and US. Note that during T256 there is a slight diminution in the peak aortic blood flow, and an increase in right ventricular pressure and heart rate (HR-CR). The painful electric stimulation (US) produced a marked increase in the amplitude of the peak blood flow, right ventricular pressure and heart rate.

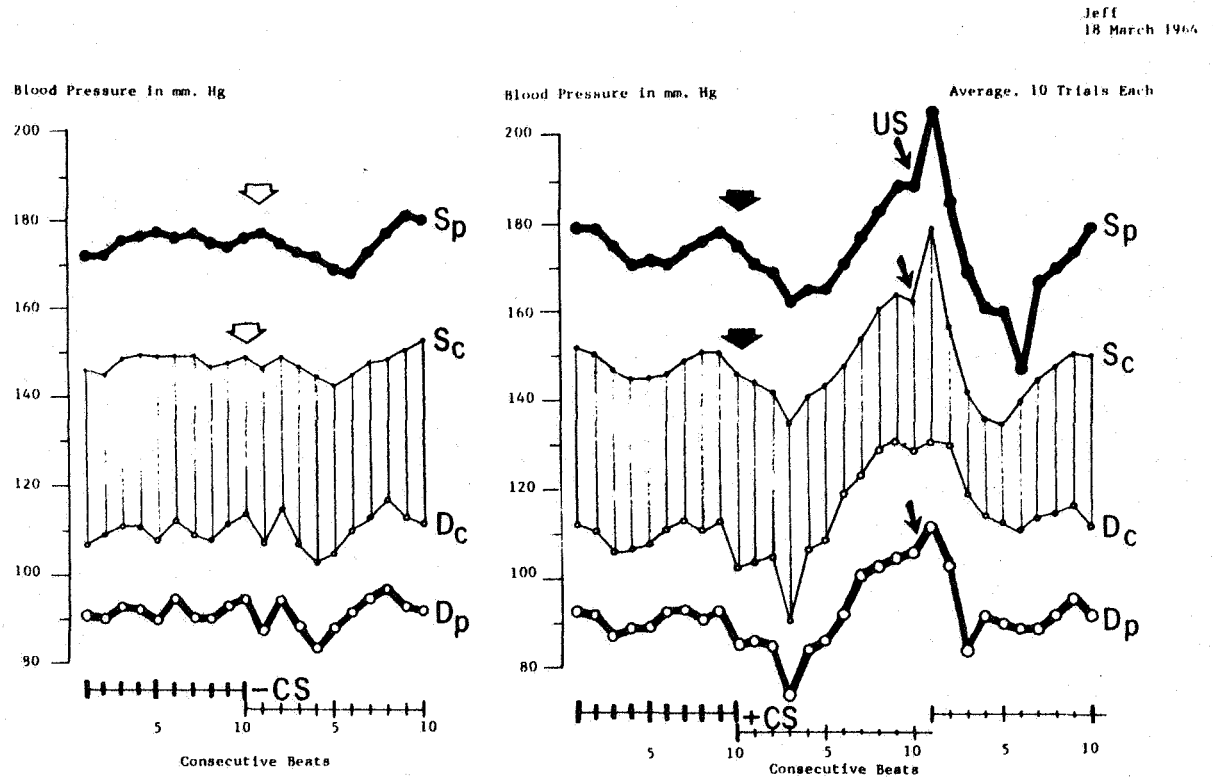

FIGURE 5: Illustrates peripheral (abdominal) systolic (S_p) and diastolic (D_p) aortic blood pressure and central (ascending aorta) sytolic (S_c) and diastolic (D_c) blood pressure recorded simultaneously during a reinforced (+CS) and a non-reinforced (-CS) conditional stimulus. Note that during the reinforced conditional stimulus there is an increase in the central and peripheral aortic blood pressure. The blood pressure response during the +CS is greater in the D_c than in the D_p but there is no difference in this response in the systolic blood pressure. Painful stimulation (US) produced an initial increase in systolic blood pressures followed immediately by a decrease and narrowing of the pulse pressure. Note that there is differentiation of the blood pressure conditional response to the -CS which was never reinforced. Measurements of blood pressures were done beat-by-beat and each consecutive beat represents a measurement of 10 pressure pulses, therefore the data summarizes 1600 individual blood pressure measurements.

HEMODYNAMIC CHANGES ACCOMPANYING VARIOUS PATTERNS OF CLASSICAL CARDIAC CONDITIONAL REFLEXES IN DOGS.

This study was designed to examine the hemodynamic changes accompanying various patterns of cardiac conditional reflexes. The intact animal was isolated in a soundproof chamber and restrained only by a leash and loose sling under the abdomen. The conditional stimulus (CS) was a six-second tone and the unconditional stimulus an electric shock to a leg of half second duration. In 12 dogs, heart rate (HR) and proximal aorta blood flow (ABF) were measured cycle by cycle, in the majority aortic pressure (BP) and in some dogs right or left ventricular pressure (VP) and pulmonary artery BP were recorded. Several patterns of HR response during the CS were encountered: (I) acceleratory, (II) biphasic, initial deceleration followed by acceleration, (III) biphasic, initial acceleration followed by deceleration, and (IV) decelleratory. During patterns (I) and (II), HR rose 40 to 80 beats per minute; there was a measurable reduction in ABF, reflecting stroke volume, during the early phase of acceleration quickly followed by return to control level or above as HR continued to increase. BP rose 10 to 30 mm Hg, the diastolic rise usually preceding the systolic. Accompanying these changes a rise of 5 to 15 mm Hg in RVP was observed. In (III), the changes were variable. In pattern (IV), ABF increased slightly and BP and systolic RVP fell, but the baseline RVP was higher than in other patterns. As in exercise, though differing in some behavioral aspects, the hemodynamic changes during conditioning obviously involve an interplay between important controlling factors, including HR, peripheral resistance, ventricular inflow and outflow.

HEMODYNAMIC CHANGES ACCOMPANYING THE OCULOCARDIAC REFLEX.

The oculocardiac reflex (Aschner-Dagnini Reflex) is a reflex of vagal origin characterized by slowing of heart rate (HR) during or following pressure on the eyeballs. The present study was designed to determine changes in aortic or inferior vena cava flow, right or left ventricular pressure (RVP or LVP) and aortic pressure (BP) accompanying the oculocardiac reflex. Four unanesthetized dogs with good oculocardiac reflexes were studied. Eyeball pressures were exerted manually. An electromagnetic flow probe was implanted chronically on the ascending aorta or inferior vena cava. BPs were measured with indwelling catheters and Statham BP transducers.

Results: Mean HR during the control period was 142 beats per minute (bpm) and during eyeball pressure the HR slowed to a mean of 75 bpm. Occasionally the HR slowed to 15 bpm during short periods of time and incomplete AV block and extrasystoles were observed. The latency of the cardiac reflex was from immediate to 2 seconds after eyeball pressure. During oculocardiac slowing ascending aortic peak flow and inferior vena cava flow increased slightly (about 5 to 10%); systolic RVP decreased slightly (4 to 9 mm Hg.); systolic LVP decreased 10 to 20 mm Hg; and systolic and diastolic BP decreased 5 to 38 mm Hg below control levels. The study revealed significant hemodynamic changes accompanying the oculocardiac reflex. The slight increase in peak flow with a decrease in LVP suggest an inotropic effect, but the slight increase in peak (pulsatile) flow could also be influenced by an increase in inferior vena cava flow.

STUDIES OF INFERIOR AND SUPERIOR VENA CAVA FLOW DURING CARDIAC CONDITIONING.

In a previous progress report we stated that the right ventricular pressure (RVP) usually increases during the acceleratory HR-CR and the systolic RVP decreases slightly during the deceleratory HR-CR. The present study was designed to determine if these increases and decreases in RVP are influenced directly by venous inflow into the heart and indirectly by the stroke volume (SV). In one dog that had a definite bradycardia as a CR, we have found that the inferior vena cava flow decreases while the RVP is also decreasing. This suggests that the decrease in RVP is associated with a decrease in inferior vena cava flow.

This preliminary data of venous inflow suggests that the parameter of venous inflow is an important one to include in the measurement of hemodynamic changes during cardiac conditioning.

One of the most difficult problems in this chronic preparation in which vena cave flow is recorded is the kinking of the superior or inferior vena cava. This has been solved by cannulating the veins with a special blood flow probe. Using this technique we have found 3 components of the vena cava flow not previously described. There is a steady baseline blood flow; 2) a negative and positive regurgitating flow and 3) a ventricular regurgitant flow. The baseline flow represents the true venous flow; the negative and positive regurgitating flow is a backward or inward sudden flow produced by auricular contractions and is usually influenced by respiration; the ventricular regurgitant flow is produced by the ventricular contractions, but it is not known at present whether there is a ventricular venous regurgitation in the dog. An ancillary part of this study is the relationship between peripheral resistance and venous flow. Preliminary data suggest that venous blood flow increases with an increase in peripheral resistance. The relationship of these hemodynamic changes are still under investigation.

OPERANT CONDITIONING OF HEART RATE IN MONKEYS

The modification of cardiac functions by the experimental control of environmental consequences within the operant conditioning framework has received increasing experimental attention in several recent research reports. Although initially unsuccessful attempts to develop an operant discrimination in humans based upon heart rate changes were reported by Mandler and Kahn (1960) and negative results were obtained by Harwood (1962) in an attempt at operant conditioning of human heart rate deceleration, Shearn (1962) did report success in the operant conditioning of heart rate acceleration in a well-controlled study with humans. And most recently, Engel and Hansen (1966) obtained positive results in conditioning heart rate deceleration with human subjects reinforced by money for heart rate slowing. Similarly, Frazier (1966) demonstrated that heart rate acceleration could be controlled as an operant avoidance response to an exteroceptive visual warning stimulus in the presence of which shocks were delivered to human subjects whose heart rate decreased below predetermined levels.

Operant conditioning of heart rate has also been reported recently by Trowill (1966) by Miller's laboratory at Yale working with intracranial electrical stimulation of the brain as a reinforcing consequence in curarized laboratory rats. Animals selectively reinforced with brain stimulation for fast heart rates showed significant acceleration whereas those reinforced for slow heart rates showed significant cardiac deceleration. In addition, convincing evidence has also emerged from Miller's laboratory to support the contention that such differential heart rate changes can be brought under the discriminative control of exteroceptive stimuli in the absence of potential movement artifacts.

The present experiment attempts to extend some of these findings with respect to the operant conditioning of cardiac functions to the rhesus monkey using positive reinforcement of heart rate change under conditions of continuous environmental control.

Seven rhesus monkeys restrained in primate chairs and confirmed in sound-attenuating isolation booths served as subjects. Following a 1 to 3 week chair adaptation period, all animals were maintained on a 24-hour food deprivation schedule and continuous recording of heart rate established baseline levels prior to initiation of the experimental manipulations.

EKG polygraph recordings were obtained from implanted stainless-steel teflon coated electrodes in the right thoracic and left inguinal region. A commercially available cardiac monitor (Melpar M100-XM1) operating from the EKG input detected heart rate changes and provided for contingent programming of liquid food (Tang) reinforcements. The cardiac monitor was equiped with a multiposition selection device which provided a signal at preset heart-rate values ranging from 20 beats per minute to 350 beats per minute in steps of 5 or 10 beats per minute. An additional control parameter of the cardiac monitor permitted programming of the preselected heart-rate signal on the basis either of a single beat-to-beat interval or two consecutive beat-to-beat intervals at a specified rate. In the latter "delayed" control position, two long heart cycles in succession at the specified preset rate were required in order to produce the programming signal which was activated after the third pulse. The output signal from the cardiac monitor provided for delivery of food reinforcement to the animal in accordance with the preprogrammed requirement for maintenance of specified heart-rate levels. The number and temporal distribution of these food reinforcements was recorded continuously on a Gerbrands cumulative recorder.

Measures of heart rate were obtained by counting the number of R waves in the EKG during 10 to 20-second time samples selected from every 2 to 5-minute segment of the continuous experimental record. A count of the number of reinforcements programmed by the cardiac monitor at each preset heart rate also provided for the measurement of cardiac change. In addition, a beat-by-beat analysis of heart rate was provided by the measurement of time intervals in msec. between R waves (HR interval). Overall estimates of operant cardiac control were also obtained by measuring the number of cardiac decelerations reinforced over a given time period. Time samples ranging between 1 minute and 9½ hours were obtained at critical stages of the experiment with all monkeys and the number of cardiac responses meeting the preset criterion required for reinforcement per hour was calculated. Statistical analysis of the data was provided by histogram distributions, standard deviations, and T-tests.

The general procedure for establishing and maintaining operant control of heart rate with the animals studied in these experiments followed closely the well-known "shaping" approach characteristically associated with operant conditioning techniques. Initial settings of the cardiac monitor provided for programming of the output reinforcement signal at heart rate values approximating predetermined baselines for each animal with accelerations above this baseline failing to produce food. As heart rate slowing increased in frequency and the number of reinforcements increased, the cardiac monitor setting was adjusted to require lower heart rate levels in order to provide the reinforcement programming signal. Adjustments of the heart rate slowing requirement were continued in progressively decreasing steps until each animal could no longer meet the criterion for reinforcement and no food was obtained over an extended period. At this point, the monitor was set to reinforce the minimum cardiac rate which could be maintained by each animal and extended samples of such operant cardiac control were obtained. The effect of free-feeding upon the heart rate level under these conditions was explored and the course of reaquisition of such operant heart rate control was investigated with several monkeys.

Six of the seven monkeys serving as subjects in the experiment showed clear evidence of operant control over both heart rate slowing and frequency of sinoauricular standstill ("droped" beats). Figure 1 illustrates the spontaneous appearance of "dropped" beats in the EKG record in a monkey before conditioning was initiated. As indicated by the arrows, this true cardiac event is characterized by the failure of an actual R wave to appear at the expected interval followed by a temporary slowing of the heart rate. Such sinus arrhythmia was usually observed during the pre-experimental baseline control period when the laboratory noise level was low and the animal was quiesent. Figures 2 and 3 illustrate the reinforcement of such "dropped" beats as programmed by the cardiac monitor on both a single beat basis (Figure 2) and the "delay" contingency basis requiring two "dropped" beats in succession (Fig. 3).

Figure 4 summarized the findings throughout the course of a continuous 4-day experiment with Monkey Bobo following application of the "shaping" procedure described above. The data is plotted in terms of the number of reinforced cardiac responses (heart rate slowing) per hour for each day as a function of the preset rate requirement on the cardiac monitor. The distribution of such reinforced responses during the first day of exposure to the indicated "shaping" procedure shows a peak at values around 155 and 160 beats per minute which approximates the predetermined baseline heart rate levels for this monkey. During the second experimental day a consistently larger number of reinforcements were obtained at a cardiac monitor setting of

110 beats per minute, and by the fourth day the animal showed a consistently high frequency of reinforcement with the required heart rate setting on the cardiac monitor at 115 beats per minute.

Figure 5 shows changes in heart rate in beats per minute for another monkey over a three-day experimental period characterized by progressively more stringent heart rate slowing requirements for food reinforcement. The data was obtained by counting the number of R waves during 10-second segments of the EKG record immediately before (heavy line) and immediately after (light line) each reinforcement programmed at the indicated setting (absissa) of the cardiac monitor. In addition to the obvious decrease in overall heart rate which developed over the three-day course of the experiment, a consistent and relatively stable difference between the pre- and post-reinforcement rates is reflected in this data plot. Characteristically, a marked slowing of heart rate occurred before reinforcement followed by a transient increase immediately following each reinforcement. Significantly, this post-reinforcement heart-rate increase did not invariably accompany the licking and drinking which followed delivery of the Tang. Despite the marked decrease in overall heart rate from approximately 180 to 100 beats per minute throughout the course of the three experimental days, however, a relatively constant difference approximating 10 to 20 beats per minute was maintained between the pre- and post-reinforcement heart rates.

Figure 6 summarizes the heart rate changes recorded for Monkey M3 throughout 17 continuous hours of a pretraining baseline control period (A), 45 continuous hours of exposure to the operant conditioning "shaping" procedure described above (B), and 10 continuous hours of "free-feeding" following the conditioning session shown in B during which reinforcement was no longer contingent upon heart rate slowing (C). During the course of the 45-hour conditioning session, heart rate can be seen to have declined from baseline values ranging around 170 beats per minute to operant controlled rates approximating 120 beats per minute. Following removal of the heart rate slowing requirement and initiation of the "free feeding" regime, the heart rate quickly recovered to baseline levels ranging around 170 beats per minute.

In summary, then, the results of these experiments indicate quite clearly that the cardiac rate of the rhesus monkey can be brought under the control of exteroceptive environmental consequences within an operant conditioning framework when delivery of a liquid-food reinforcement is made contingent upon "droped" beats or heart rate slowing. Although the exact nature of the physiological processes participating in such operant cardiac responses is far from clear, the fact that heart rate slowing and sinus arrhythmia consistently followed selectrive reinforcement of "dropped" beats in our experiments suggests control through central nervous system or vagal effects upon the heart. Of course, the role of respiration in mediating at least some of these responses must not be overlooked, although it seems doubtful on the basis of Trowill's findings with curarized rats ventrilated at a constant rate that respiratory rate per se can be implicated as the critical factor. Measurements of tidal volume and pulmonary alvealor exchange, presently difficult to obtain in the unanesthetized monkey, may shed some light on the respiratory mechanisms involved. Finally, of course, the role of musculo-skeletal inhibition in the mediation of the conditioned cardiac slowing described in these experiments is difficult to assess. On several occasions in the course of these studies, however, heart rate slowing was recorded in the presence of concurrent licking and drinking behavior.

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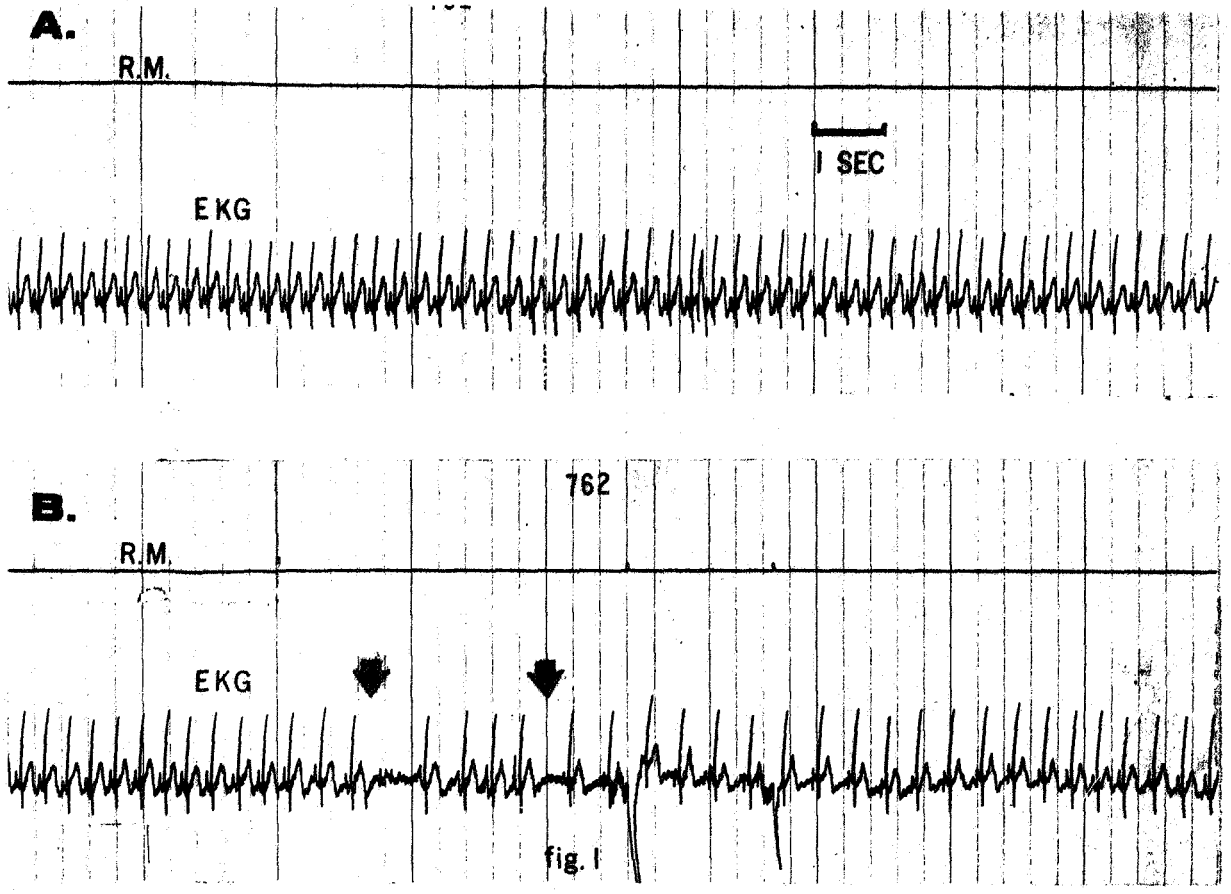


fig. 1

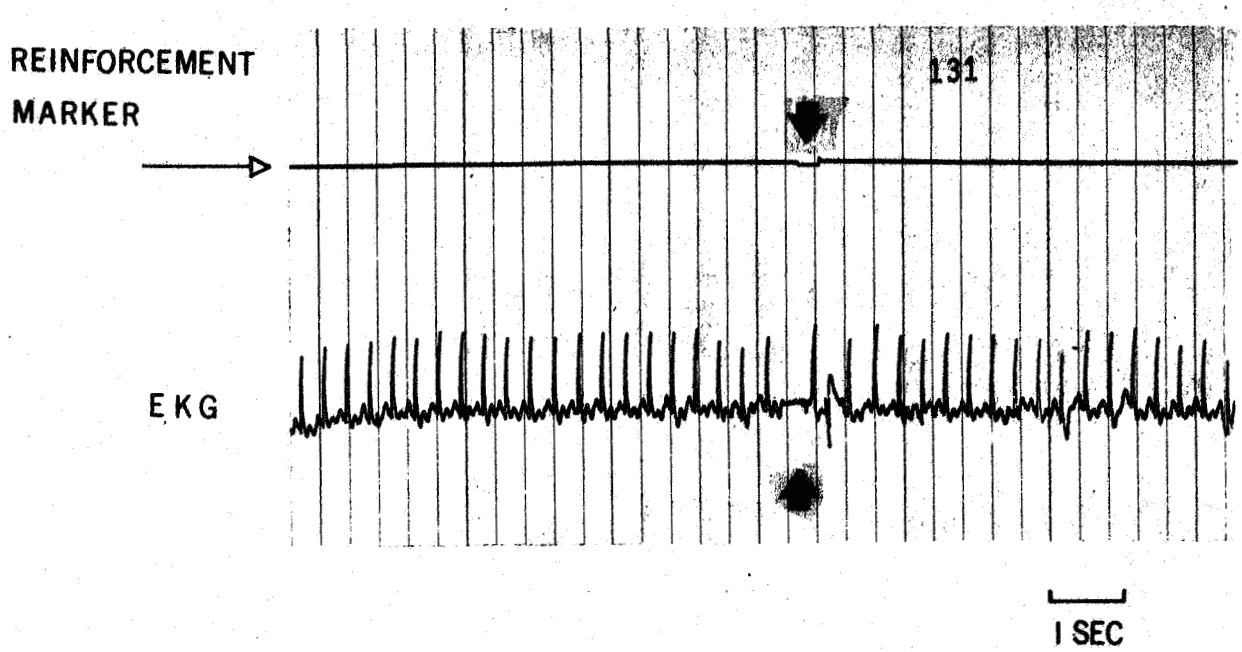


fig. 2

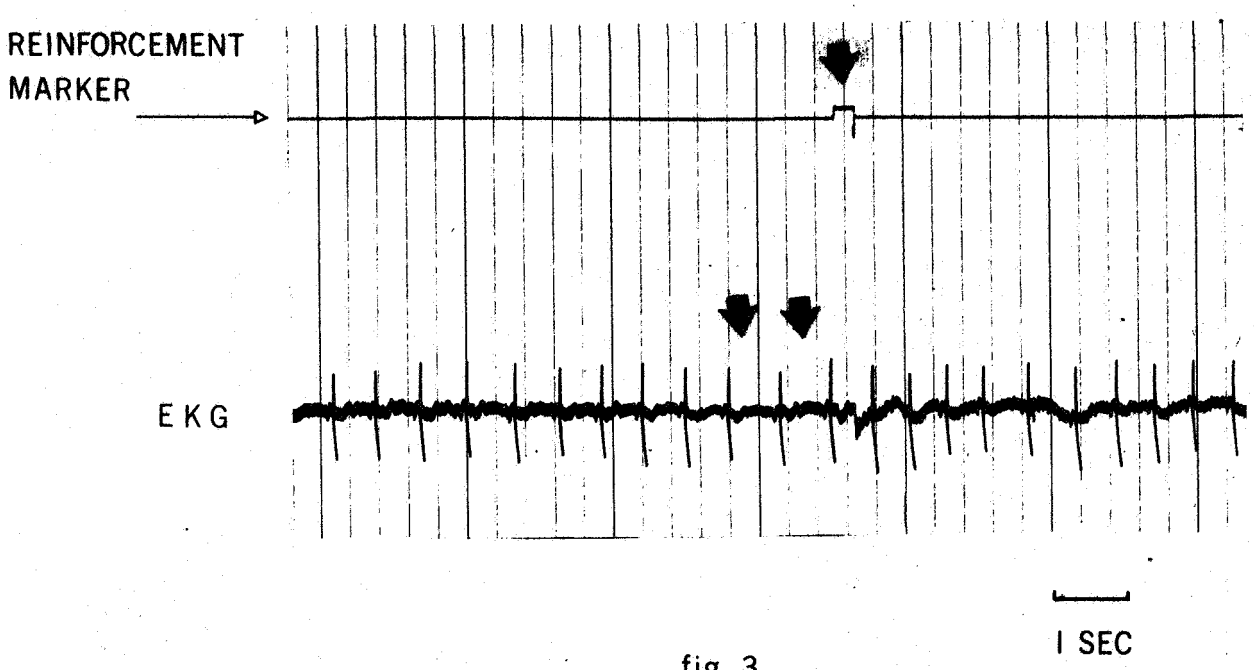


fig. 3

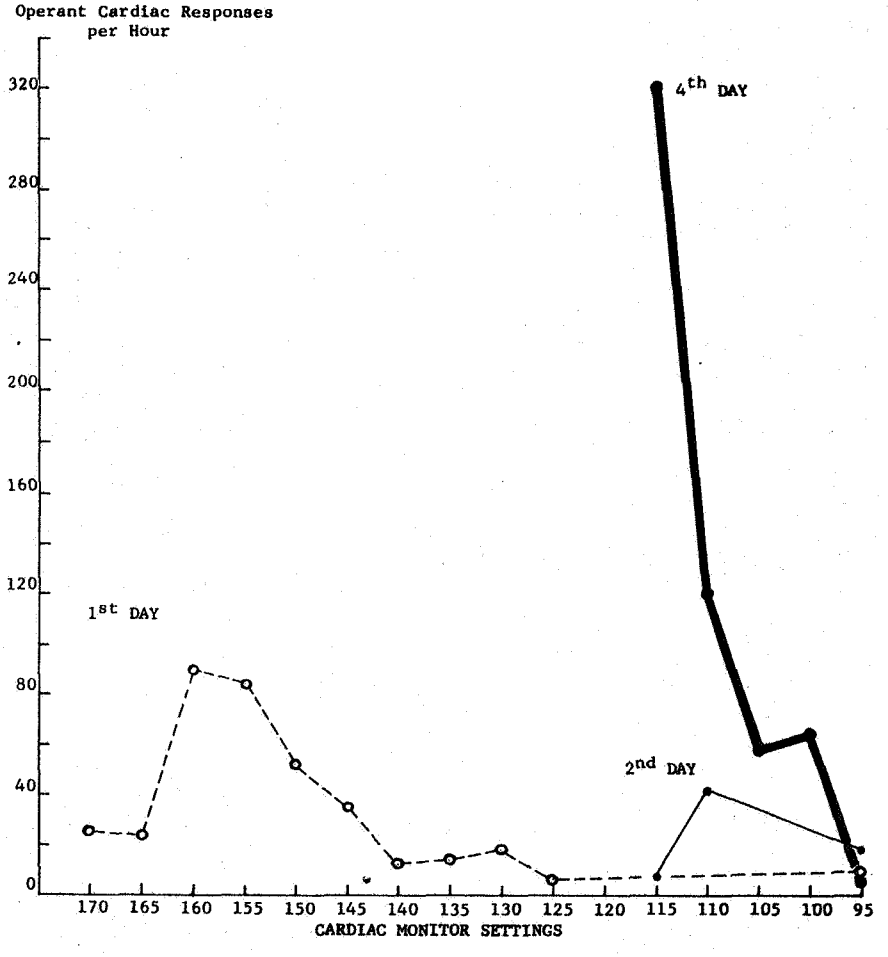

Operant Cardiac Responses
per Hour
320
280
240
200
160
120
80
40
0
4th DAY
1st DAY
2nd DAY
170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95
CARDIAC MONITOR SETTINGS

FIGURE 4

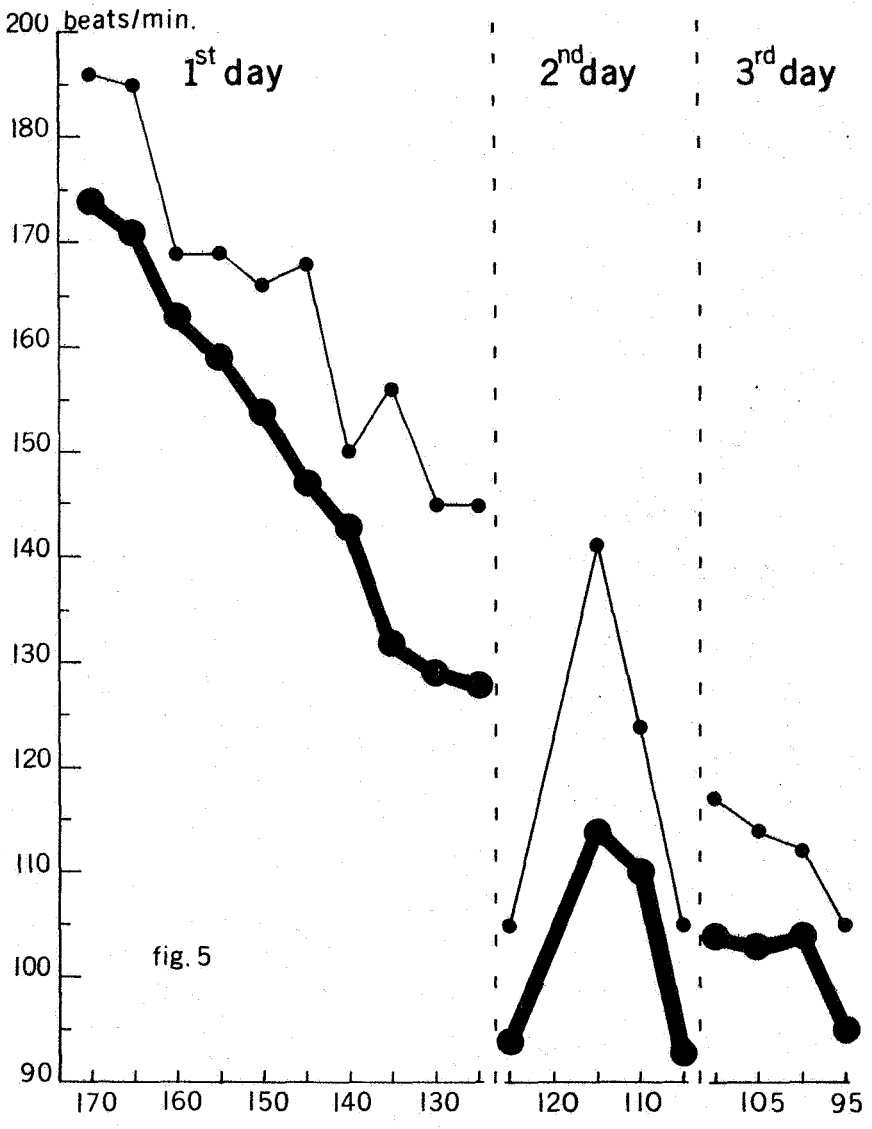

200 beats/min.
1st day
2nd day
3rd day
180
170
160
150
140
130
120
110
100
90
170
160
150
140
130
120
110
105
95

fig. 5

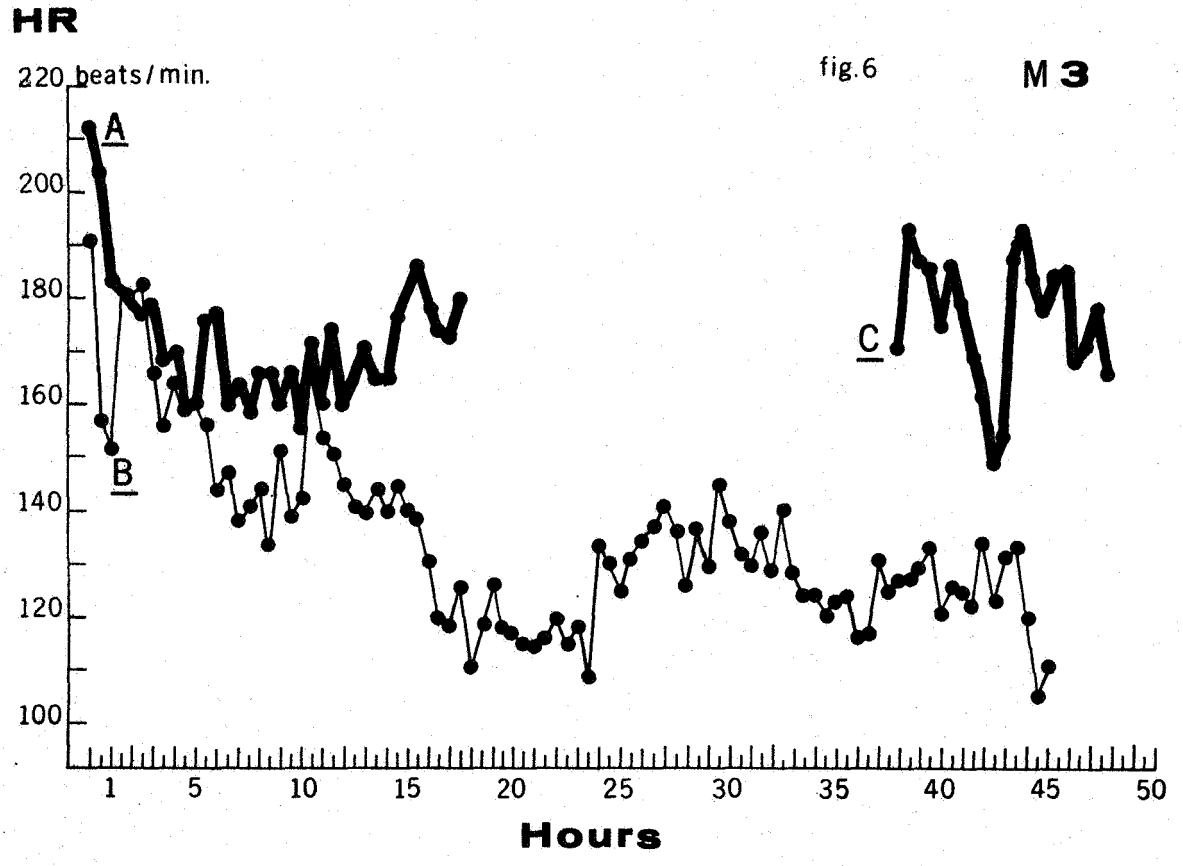

HR
220 beats/min.
A
B
C
200
180
160
140
120
100
1
5
10
15
20
25
30
35
40
45
50
Hours

fig.6 M 3

MEASUREMENT OF HEART RATE IN PRIMATES

Measurements of heart rate have been done in 20 monkeys. Several methods for evaluating heart rate have been used. We have used the most common method of counting heart rate, that is, counting the number of R waves from the electrocardiograms in segments of 6 or 10 seconds. The counts are usually done by hand and collection of data is usually very tedious. This method is useful in determining heart rate changes due to stress and to stimuli in the environment. Due to the enormous amount of heart-rate data collected in a 24-hour period this method is very time-consuming.

For this reason, another method to measure heart rate was developed. In this second method, the R wave was used to activate a triggering unit, which in turn activated a Gerbrans cumulative recorder. The Gerbrands recorder was reset automatically with a precision digital counter which had been set to put an output reset voltage every minute. Figure 1 shows an actual cumulative Gerbrands heart-rate record from a monkey. This method is useful in measuring heart rate for prolonged periods of time ranging from 12 to 24 hours of continuous recording. Figure 2 illustrates an example of the sensitivity of this method in experiments where the person was used as a stimulus in the environment.

Another method which we have used, employs a Gilford cardiotachometer to count heart rate beat-by-beat. The Gilford cardiotachometer is triggered by the R wave using a triggering unit described elsewhere (J. Exper. Anal. Behav., Vol. 6, pp. 61-64, 1963). Figure 3 illustrates beat-by-beat changes in heart rate from the Gilford cardiotachometer.

A fourth method which is being used now, consists of measuring the R to R interval with a Wirtschafter ruler. Beat-by-beat measurements are obtained with this ruler in beats per minute. Using this method, we have obtained heart-rate distribution patterns in four monkeys, as shown in Figure 4.

FIGURE 1

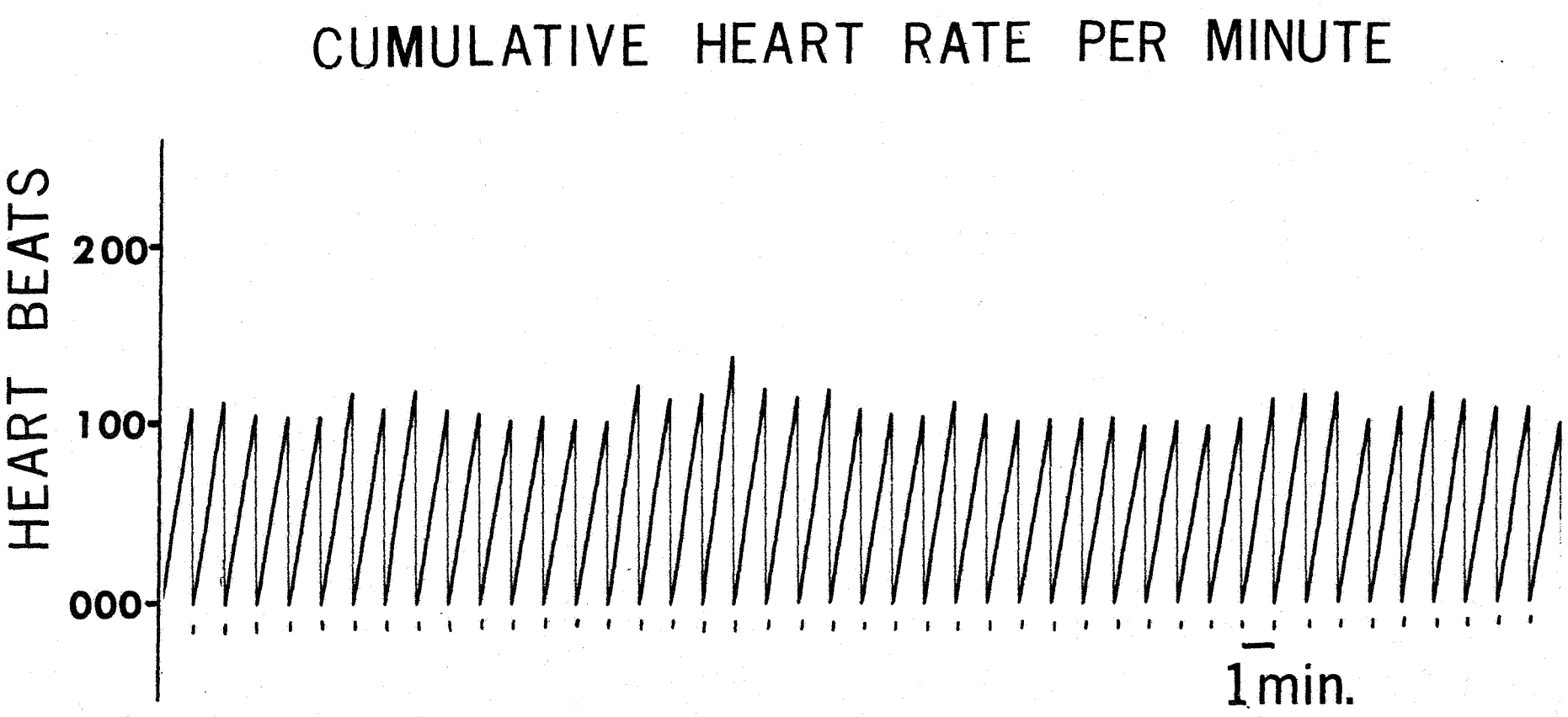


Figure 1: Cumulative Gerbrands heart rate record from an undisturbed monkey. Ordinate indicates heart beats. Heart beats are accumulated in the slope line. The Gerbrands recorder is reset every minute with a precision digital clock. The abscissa indicates the time in minutes. Note that the heart rate fluctuates between 100 - 120 bpm. This type of recording of heart rate is useful in chronic experiments in which measurements are done continuously for 24 hours.

FIGURE 2

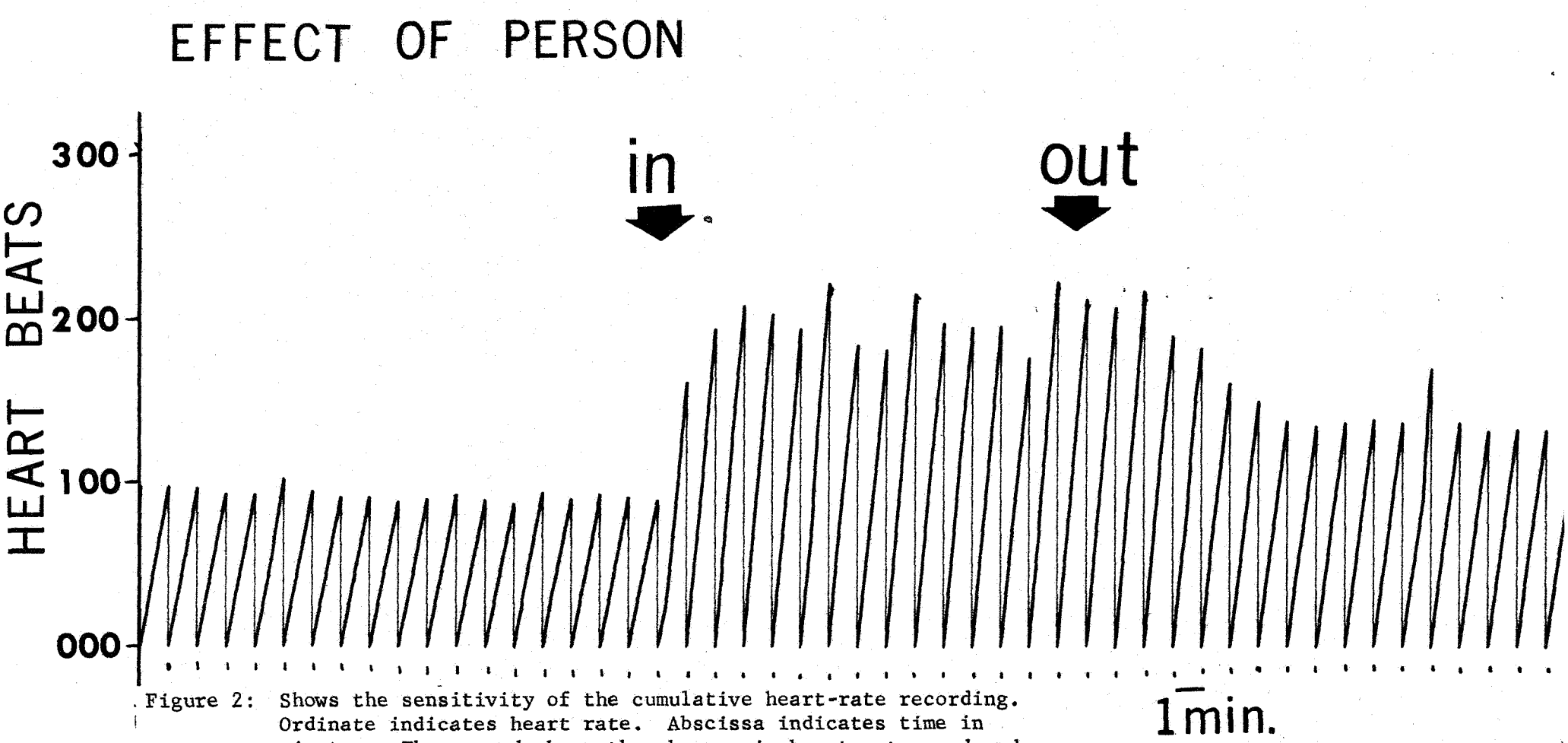


Figure 2: Shows the sensitivity of the cumulative heart-rate recording. Ordinate indicates heart rate. Abscissa indicates time in minutes. The record shows the changes in heart rate produced by a person entering (in) and leaving (out) the room. The heart rate changes from about 95 to 200 bpm when the person enters the room. When the person leaves the room the heart rate decreases in the next 5 minutes to a new heart rate level of 125 bpm. Return to the baseline can take from 15 to 30 minutes. The control period in this tracing was taken when the animal was very quiet and undisturbed. This type of response can be useful to determine psychocardio-vascular reactivity in monkeys.

Figure 3: This tracing indicates: (A) blood pressure from the lower abdominal aorta and (B) heart-rate changes beat-by-beat from a Gilford cardiotachometer. At A, the upper portion indicates systolic pressure and lower indicates diastolic pressure. Systolic pressure was around 150 mm. Hg. Diastolic blood pressure was around 86 mm. Hg. At B, the pre-stimulus heart-rate level is about 130 beats. Blood pressure and heart rate were recorded simultaneously in this experiment. The middle portion of the record (in - out) indicates the effect of person on both blood pressure and heart rate in a monkey. Note that the systolic blood pressure change above pre-stimulus level is about 50 mm. Hg for the systolic pressure and about 25 mm. Hg for the diastolic pressure. The heart-rate changes during the same period were about 100 beats above pre-stimulus heart-rate levels. Time is indicated in the abscissa. Deflections in the cardiotachometer tracing indicate artifacts due to movements.

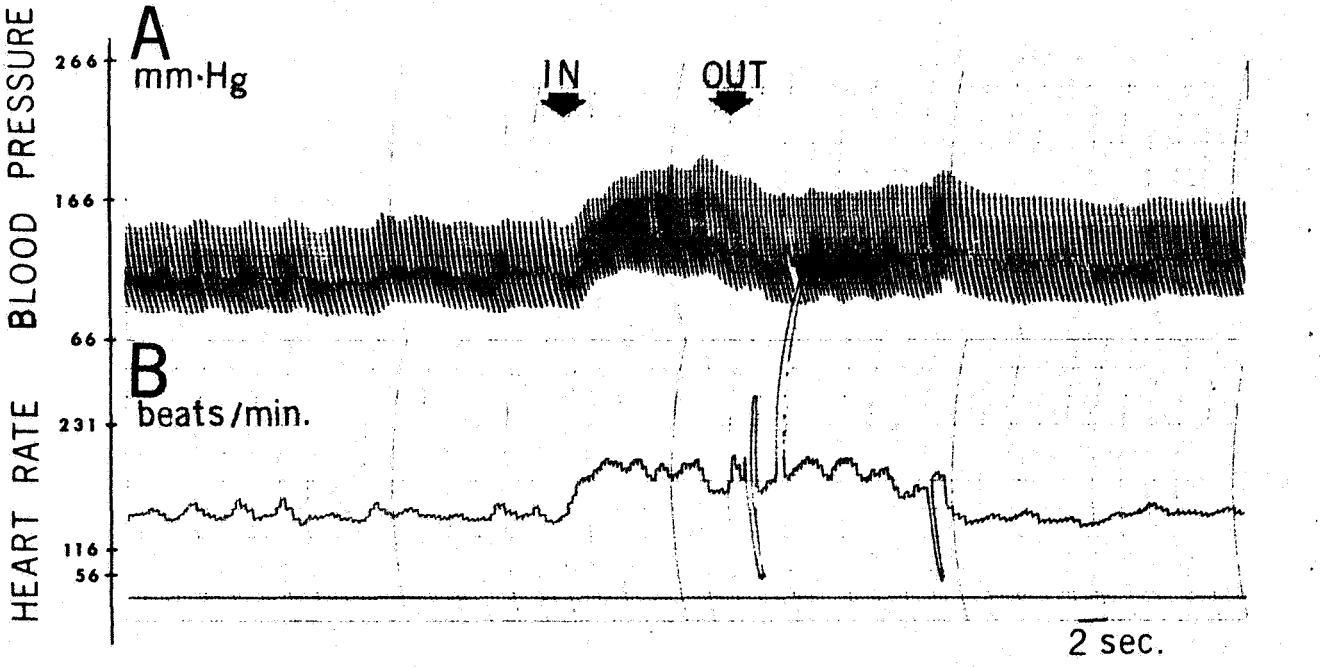
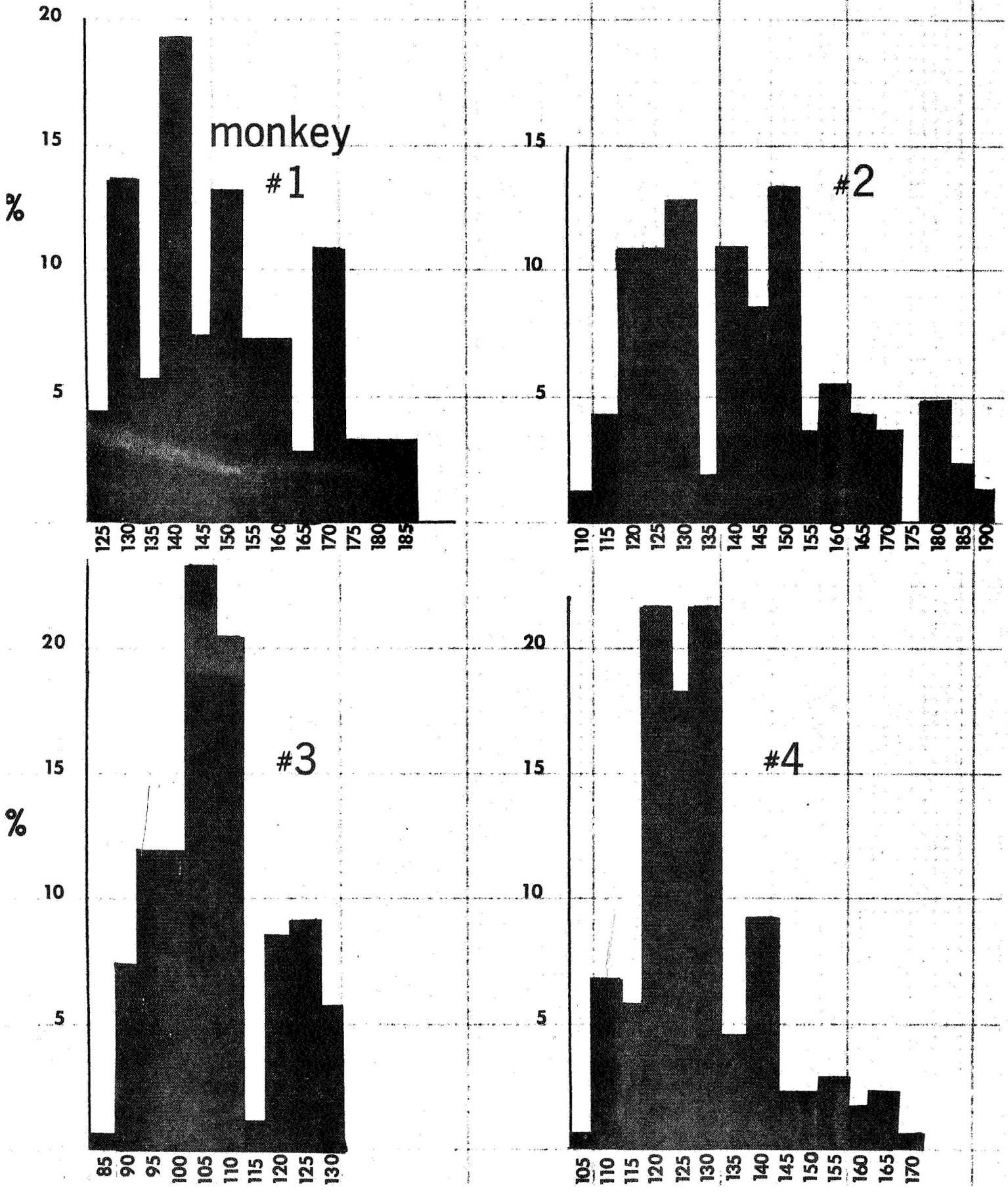


Figure 4: Heart-rate histograms in four monkeys. Ordinate is the incidence in percentage of a given heart-rate distribution. The abscissa is heart rate in increments of 5 beats. Monkey #1 shows a distribution of heart rate between 125 and 185 bpm with a peak distribution around 140 bpm. Monkey #2 shows a distribution of heart rate between 110 and 190 bpm. There is a double-peak distribution at 125 and 150 bpm. Monkey #3 shows a distribution of heart rate between 85 and 130 bpm with a peak distribution around 105 bpm. Monkey #4 shows a distribution between 105 and 170 bpm. There is a double-peak distribution at 120 and 130 bpm. Smoothing of the distribution can be accomplished by using increments in the abscissa between 10 to 20 beats. The heart-rate histogram information in monkeys resembles similar data obtained in dogs. Heart-rate distribution may be useful in studying periodicity patterns and the effect of weightlessness on heart rate. In normal heart-rate distribution, there is a tendency of a maxima which is shifted slightly to the left. Excitement, fear and other emotional reactions usually have a tendency to shift this maxima to the right.

HEART RATE HISTOGRAMS

FIGURE 4



CHRONIC MEASUREMENT OF THORACIC AND ABDOMINAL AORTIC BLOOD PRESSURES IN UNANESTHETIZED DOGS WITH A RING-CATHETER TECHNIQUE.

Blood pressure in chronic experiments in dogs has been determined by direct arterial puncture of a superficial artery (1,2,3,4,5,6); by the ausculatory (occlusion) method (7,8,9,10,11,12,13,14,15); by an electronic transducing circuit wrapped around an artery (16); by an externalized arterial loop (17,18); and by a blood pressure sensor attached to an aortic arterial graft (19).

The disadvantages of direct arterial puncture are that a very well-trained and cooperative animal is required and that daily punctures can produce thrombosis, hematoma or painful areas. The auscultatory method, which eliminates the difficulties inherent in daily arterial puncture, gives readings of blood pressure at intervals varying between 10 to 30 seconds, depending on the velocity at which the pressure in the occlusion cuff is released. The auscultatory method cannot be used when instantaneous beat-to-beat changes in blood pressure are measured. In the third method, employing extra-arterial, transducing-electronic circuitry, the calibration of the blood pressure pick-up, which is usually attached around the artery, is very critical, and blood pressure measurements are relative unless they are compared with a direct arterial reading.

Because direct arterial puncture provides accurate and objective measurement of blood pressure and also describes beat-by-beat blood-pressure changes, the present technique was developed. In order to eliminate the daily puncture, a catheter was inserted into the artery and left in place for months. In the initial phase of these studies, which were begun about five years ago, several types of tubing, polyethylene and polyvinyl, were used. The polyvinyl tubing was finally selected, because constant flushing with heparin was not necessary and kinking of the tubing was unlikely to occur. Through trial and error, we found that intra-arterial catheters inserted into the arteries without attachment to surrounding tissue were usually rejected or pulled out accidentally. Initially, rings for attaching the catheter to the artery were built into the catheters by heating the surface of the tubing with a soft flame, but this treatment usually weakened the wall of the tubing. Later it was found that rings could be flued to the tubing with a non-toxic adhesive (Monomer 910 from Eastman). Thus the final innovation in the construction of the tubing was named the ring-catheter technique.

Methods and Materials

Thirty-five dogs weighing from 5 to 32 kg were used. Abdominal aortic blood pressures from the lower third of the aorta were recorded in twenty-five dogs. Thoracic blood pressures from the upper third of the aorta were recorded in five dogs. In the remaining five dogs, abdominal and thoracic aortic pressures were recorded simultaneously with individual catheters inserted through the common carotid into the thoracic aorta close to the orifice of the brachiocephalic artery and through the femoral artery into the lower third of the abdominal aorta above the trifurcation. All abdominal aortic catheters were inserted through the femoral artery. Thoracic aortic catheters were inserted through the common carotid or the brachial artery.

All blood pressures were obtained in unanesthetized dogs in the standing position, restrained slightly by a leash and a small sling on the back. Pressures were taken while the dogs were alone inside a 7 x 7 x 7 ft soundproof room with an 80 db sound attenuation. The temperature inside the room was thermostatically controlled to 74° F.

Direct aortic blood pressures were measured with a P23De Statham strain-gage blood-pressure transducer, an electromechanical device which transduces minute displacements to proportional resistance changes. The blood pressure transducer was taped on the thorax of the dog at the level of the heart. The nominal sensitivity of the strain-gage circuit was 100 μv (open circuit) per volt input per cm of mercury pressure. Volume displacement was 0.04 cubic mm per 100 mm Hg pressure. The output from the blood pressure transducer was coupled to an Offner strain-gage coupler and amplifier. A curvilinear Offner-Beckman, type R, transistorized polygraph was employed to record the blood pressure.

Construction of Catheters

Various types of polymeric plastic tubing materials were used, such as polyvinyl tubing (surco clear from Surprenant Mfg. Co., Clinton, Mass.), Resinite Hi heart 105 C vinyl (Borden Chemical Co., Compton, California) and Temflex medical tubing (Minnesota Mining Mfg. Co.). The first type of polyvinyl has been used more frequently than the others.

The catheters were cut in sections of about 2 to 4 ft. The dimensions of the tube were: inner diameter 0.038 in. and outer diameter 0.070 in. Small rings, varying in width from 1 to 2 mm, were cut from a piece of tubing with the internal diameter slightly greater than the outer diameter of the blood-pressure tubing. Two or three rings were inserted and placed 2 to 4 mm apart, 10 to 20 cm from one end of the tubing (fig. 1). One or two drops of methyl 2-cyanoacrylate monomer adhesive (Eastman 910 Monomer) were placed between the rings so that the adhesive spread on both sides (see fig. 2). Since excessive application of the adhesive usually weakened the wall of the polyvinyl tubing, any excess was carefully cleaned with absorbent paper. The catheters were dried for at least 1 hour. Catheters with rough surfaces were eliminated. Catheters were tested by filling them with saline, clamping one end and applying pressure with a 20 cc syringe. Leaks in the tube were usually detected through this method.

After the catheters were implanted, they were occluded with a terminal plug, which was inserted in the end opposite to the arterial implantation. The terminal plug was prepared by cutting or filing off the head of an 18-gauge stainless-steel needle at the base and flattening and polishing the bevel end. Grip-ivory dental cement (manufactured by L.D. Caulk Co., Milford, Delaware) was put inside the bore of the needle and at the base was molded to a round end until it hardened. This type of terminal stainless-steel plug is very useful because it is easy to grasp with the fingers and does not corrode.

Surgical Procedure for Implanting the Ring-Catheter

All animals were anesthetized with sodium pentobarbital, 30 mg/kg, and surgery was carried out under aseptic conditions. Figures 3, 4 and 5 illustrates the surgical steps for implantation of a ring-catheter. For abdominal aortic placements, the femoral artery was palpated and a longitudinal incision of the skin was made. After the artery was exposed by using blunt dissection, the femoral fascia was cleaned carefully with a hemostat. Two double-0 silk threads were placed below the artery, one distal and one proximal, to control any possible accidental bleeding; these threads were removed after the ring-catheter was fixed to the artery.

Next, the end of the ring-catheter to be inserted was trimmed to the length necessary to place it into the distal aorta. The tip was cut slightly tapered (30° to 45°) to facilitate introduction into the artery after arterotomy. The flat end of a surgical knife handle No. 3 was placed below the artery as a support. An arterotomy was performed using a stainless-steel ½ circle-curve cutting-edge needle No. 1834-4 (see Figure 3). Arterotomies with iris scissors were usually too big; for this reason a cutting-edge needle was preferred. The ring-catheter was carefully inserted through this small cut. In most instances, this was the most delicate part of the surgical procedure. If a proper cut was made with the cutting-edge needle, no bleeding occurred and blood continued to circulate through the femoral artery while the ring-catheter was in place. If the cut was too wide, bleeding around the catheter was profuse and necessitated ligation of the femoral artery. Ligation of the femoral artery, in dogs, has been well tolerated and we have never seen any deleterious effects from this procedure.

Once the ring-catheter was inserted properly through the small cut in the artery, it was passed slowly until the rings came close to the opening in the artery (see fig. 4). A double-0 ligature was passed between the two rings in the catheter and tied gently around the tubing (fig. 5). One side of the silk thread was passed counterclockwise below the artery; the other was passed clockwise without occluding it, and a square knot was made. The ligature was prolonged and a knot placed about 1 cm farther up the tube, so that another tie could be placed above the upper ring; and the previous procedure was repeated. This procedure stabilized the position of the catheter in relation to the artery. Further threads were used to fasten the ring-catheter to the surrounding muscular fascia. The fascia around the artery was closed with interrupted sutures. Before the skin was closed, a lead-way section of the tubing was placed in the form of an "S" to allow the movement and possible pulling of the tubing in the chronic state.

A twelve-inch, flexible probe, which had an eye at one end and a smooth tip, and to which the loose end of the polyvinyl tubing was attached, was passed subcutaneously from the femoral region of the neck. The tubing was brought out to the surface of the neck through a small surgical wound. Figure 6 shows two methods of anchoring the tubing to the neck. At a, where the tube protruded at the neck, it was fastened by adding one or two rings and it was attached to the skin with a single suture. At b, a 1.5 cm incision was made and the rings were fixed to the underlying muscle; the remainder of the tube protruded through a small opening which was made about 1 cm above the perpendicular to the 1.5 cm incision. The wound was closed and a loop suture was placed around the tube. The external portion of the ring-catheter, which protruded from the neck, was rolled and taped with masking tape. An elastic bandage, 4 inches wide, was wrapped around the neck to cover and protect the tubing. Permacel waterproof tape or white medical adhesive tape was then put on top of the ace bandage.

Flushing Procedure

Prior to implantation into the artery, the polyvinyl ring-catheter was filled with an anticoagulant (heparin, 1000 USP units/ml--10 mg/ml). The volume necessary to flush the tubing was determined during the construction of the catheter.

Routinely during flushing, bright blood was allowed to come back through the tubing after removal of the terminal plug. Any possible clots were trapped in a disposable syringe, which could be used for applying negative pressure. After this, the catheter was flushed first with normal saline and then with heparin solution. Extreme care was taken to eliminate any bubbles in the tubing or in the transducer during the recording of blood pressure.

In order to avoid damage to the tubing during daily flushing, a soft-tip hemostat was employed to clamp the tubing. The soft-tip of the hemostat was built by inserting a piece of rubber tubing on each end or by wrapping the ends of the hemostat with one or two layers of adhesive tape.

At the end of the experiment, the catheter was always filled with heparin. An important precaution to avoid the return of blood into the catheter before closing it with the terminal plug was to clamp the tubing while it was being flushed with heparin.

We found that some catheters could be left unattended for periods ranging from one week to one month. Routinely, no flushing was done during weekends. In some cases where flushing was done every week, a stronger heparin solution (20,000 USP units/ml) was employed. This strength of heparin was used with caution and only enough to fill the catheter was injected.

Calculations of Blood Pressure

The blood pressure was manually determined by measuring the peak of the pressure wave as the systolic pressure and the trough of the pressure wave as the diastolic pressure. The Statham transducer was calibrated daily with a mercury manometer and all readings were recorded in mm Hg. Individual pressure pulses were analyzed in each dog for systolic and diastolic pressures. All peak systolic pressures were averaged. All diastolic pressures corresponding to each individual peak systolic pressure were also averaged. The blood pressure values shown in our data represent the arithmetic mean of individual peak systolic pressures and individual diastolic pressures. The individual or total averages make no reference to the true mean arterial pressure which is determined by integrating the arterial pulse waves or by techniques described elsewhere (20). Pulse pressures were computed by subtracting the individual average diastolic pressure from the individual average systolic pressure.

The reliability of the method was assessed using techniques described elsewhere by Yanof (21). Figure 7 illustrates the reaction of the manometer system to dynamic influences using Frank's technique to generate oscillations in the transducer-catheter system. Note that the duration of one complete damped oscillation is directly proportional to the length of the catheter. The frequency response of the system is inversely proportional to the length of tubing. These relationships are valid provided the radius of the catheter remains constant.

Results

Direct aortic blood pressures were measured over periods of two weeks to twelve months.

Table I summarized the values of adbominal aortic blood pressures in thirty dogs. A total of 11,390 determinations were made. The average abdominal systolic blood pressure was 167.9 mm Hg. The average abdominal diastolic pressure was 91.0 mm Hg. The individual abdominal aortic systolic pressures ranged from 138.0 mm Hg to 199 mm Hg, while the individual abdominal aortic diastolic pressures ranged from 67.0 mm Hg to 114.0 mm Hg. The pulse

pressure in the abdominal aorta ranged from 54 mm Hg to 115 mm Hg.

Table II summarized the thoracic aortic blood pressures in 10 dogs. A total of 3,232 determinations were made. The average thoracic aortic systolic pressure was 156.2 mm Hg. The average thoracic aortic diastolic pressure was 103.5 mm Hg. The individual thoracic aortic systolic pressures ranged from 121.0 mm Hg to 200.0 mm Hg, while the individual thoracic aortic diastolic pressures ranged from 78.0 mm Hg to 147.0 mm Hg. The pulse pressure in the thoracic aorta ranged from 35 mm Hg to 72 mm Hg. Two dogs showed unusually high thoracic aortic blood pressure (see dogs No. 9 and 10). Dog 9 was an old dog (approximately age: 13 years) and Dog 10 was a puppy.

Figure 8 illustrates simultaneous thoracic (A) and abdominal (B) pressures recorded in a dog with catheters of exactly equal length. Note that the average systolic pressure (158.6 mm Hg) in the thoracic aorta. In this tracing the average diastolic pressure in the abdominal aorta is slightly lower (2 mm Hg) than in the thoracic aorta, but in the majority of determinations the average thoracic diastolic pressure is usually much higher than the abdominal aortic pressure as shown in Table I and II.

The pattern of the pressure wave varied in some dogs, as shown in Figure 9. In placements close to the renal arteries, two systolic peaks (S) were usually observed (S_1 and S_2), as shown in Figure 9A and B. In placements close to the trifurcation of the aorta, the dicrotic notching was more prominent and at this level double systolic peaks were not commonly seen, as shown in Figure 9C and D. This configuration of the pressure pulse wave with two systolic peaks was not an artifact, as it was observed consistently for several weeks and was not altered by flushing.

Clotting occurred less with polyvinyl tubing because the tubing itself did not react actively with body fluids. Four to nine months after chronic implantations of some catheters, it was found that the polyvinyl tubing changed from a clear, crystal color to a whitish, milky color and that its surface was extremely soft and smooth.

Discussion

The value of abdominal aortic blood pressures were similar to those obtained by other investigators using a direct puncture of the femoral artery. Kolls and Cash (2) reported an average blood pressure of 165/60 mm Hg (range 212/104 to 120/28) in 12 unanesthetized dogs. Hamilton, et al, (22), using an optical hypodermic manometer, reported an average blood pressure of 180/89 mm Hg in 215 street dogs (range 275/140 to 100/30).

Our investigations, however, have disclosed (a) that the thoracic systolic pressures were lower than abdominal systolic pressures and (b) that the thoracic diastolic pressures were usually, but not always, higher than the abdominal diastolic pressures. Similar findings have been reported by Hamilton and Dow (23), who have explained this phenomenon in terms of superimposition of reflected waves upon the fundamental pulse form.

One definite advantage of our technique was that the direct puncture and its complications during daily recording were eliminated. Another major difference was the isolation of the animals in a soundproof room without the presence of the experimenter while measuring blood pressures.

Our technique differs from the technique of chronic catheterization used by Herd and Barger (24) in that the arterial wall is perforated only once by a cutting surgical needle rather than a double perforation with a hollow needle. Herd and Barger also mentioned the use of a single cuff, bonded to the catheter with a minute drop of cyclohexanone, to which a 5-0 suture was attached. They did not report measurements of blood pressure in dogs. They reported maintaining catheters open for 1 year. Fixation rings or "bubbles" in teflon tubing have been used previously by Gaertner (25) in intravascular catheters implanted chronically in humans and dogs for chemotherapeutic infusions.

In unanesthetized monkeys restrained in monkey chairs, Werdegar (26) and Forsyth, et al (27), using an automatic, arterial-infusion pump method, have reported continuous measurement of direct arterial blood pressures. Infusion pumps for flushing arterial catheters have been employed previously by Lehman (28) to prevent clotting in the catheters. The infusion pump technique is useful in studies where anticoagulant agents interfere with the determination of minute amounts of neurohumoral or biochemical substances. We have found that it is not necessary to flush the catheters with a pump when proper flushing techniques are employed. In experiments in monkeys, in which we have applied this technique, arterial catheters could be flushed weekly with heparin without clotting.

The configuration of the pressure pulse wave has been studied previously by Otto Frank (29), Ryan, et al (30) and many others. Recently, Thijs and Knoop (31) have found that the pattern of the pressure pulse wave can be altered by epinephrine or acetylcholine. In our experiments, pressure pulse waves from abdominal catheters, the tips of which were located below the renal arteries and about 2.5 cm above the trifurcation of the aorta, showed double systolic peaks. Pressure waves from abdominal catheters, the tips of which were placed close to, or 2 cm above, the trifurcation, usually showed smooth systolic peaks and accentuated dicrotic notches.

In one of the techniques for measuring direct blood pressure (32), the polyethylene tubing was sealed by burning the end with a flame. This practice is safe with polyethylene tubing but it is hazardous with other plastics which produce toxic fumes (33). The use of the metal terminal plug eliminates this possible hazard.

This technique has been successful in measuring aortic blood pressures in unanesthetized dogs over extended periods in experiments involving conditioning of cardiovascular responses (34) and high blood pressure produced by hypothalamic self-stimulation (35, 36). It has been especially useful in studying rapid changes in blood pressure during various types of sinus arrhythmia (37, 38). It has also been employed with rhesus monkeys where blood pressures have been under observation for several months.

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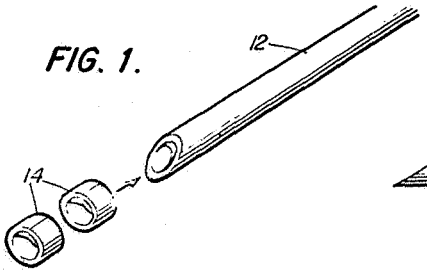

FIG. 1.

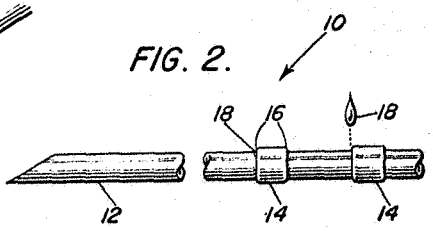

FIG. 2.

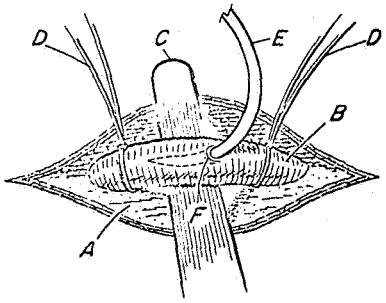

FIG. 3.

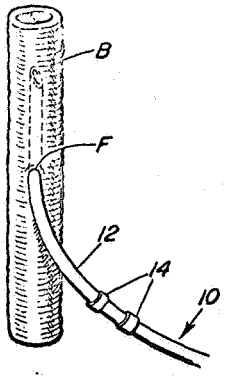

FIG. 4.

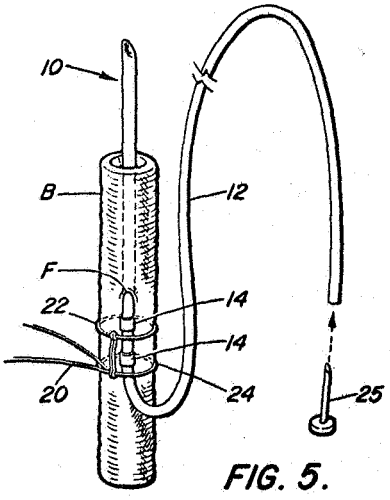

FIG. 5.

Figures 1, 2, 3, 4, 5. Preparation of ring-catheter and surgical steps for its implantation into the aorta.

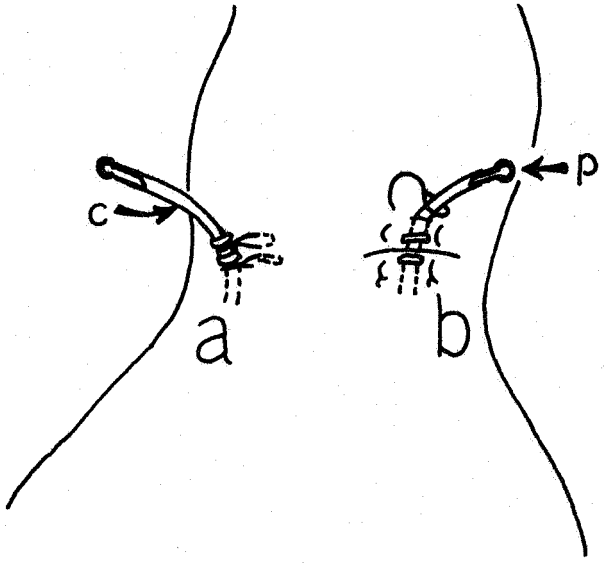

FIGURE # 6

Figure 6. Methods for fixing the ring-catheter to the neck.

a. rings outside the skin attached with a suture.
b. rings under the skin fixed to muscular fascia.

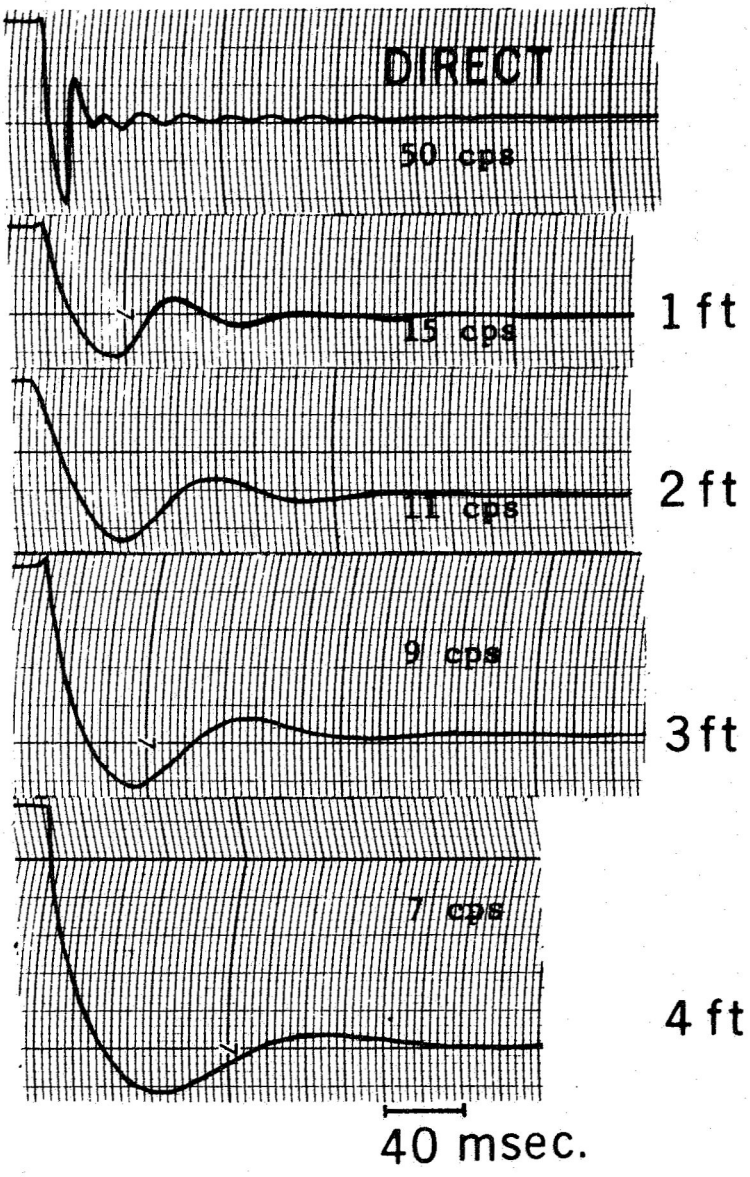

Figure 7. Frequency response analysis of transducer-catheter system. Individual curves from top to bottom indicate oscillations from transducer plus several lengths of tubing. Frequency response is in cycles per second (cps).

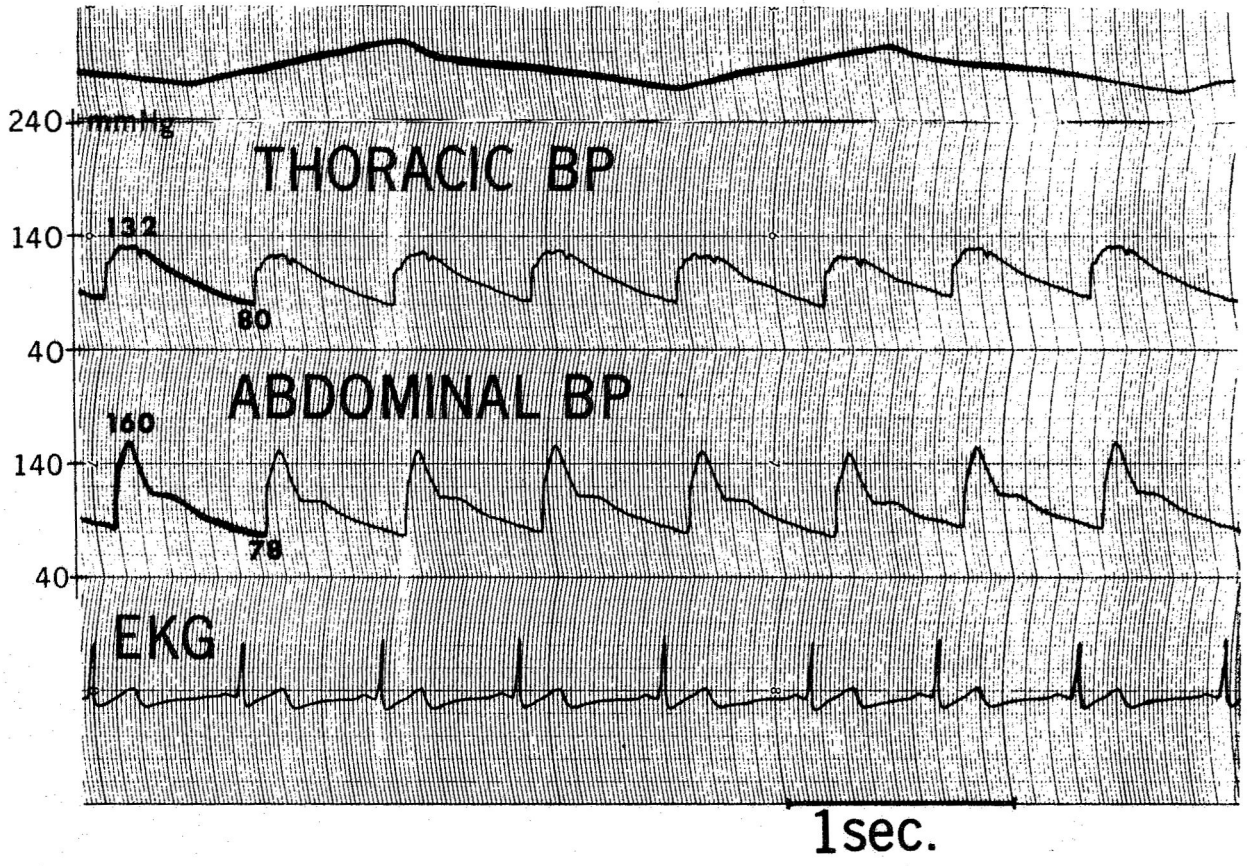

Figure 8. Tracing illustrating thoracic and abdominal aortic pressures recorded simultaneously in the same dog with a ring-catheter of equal length and diameter.

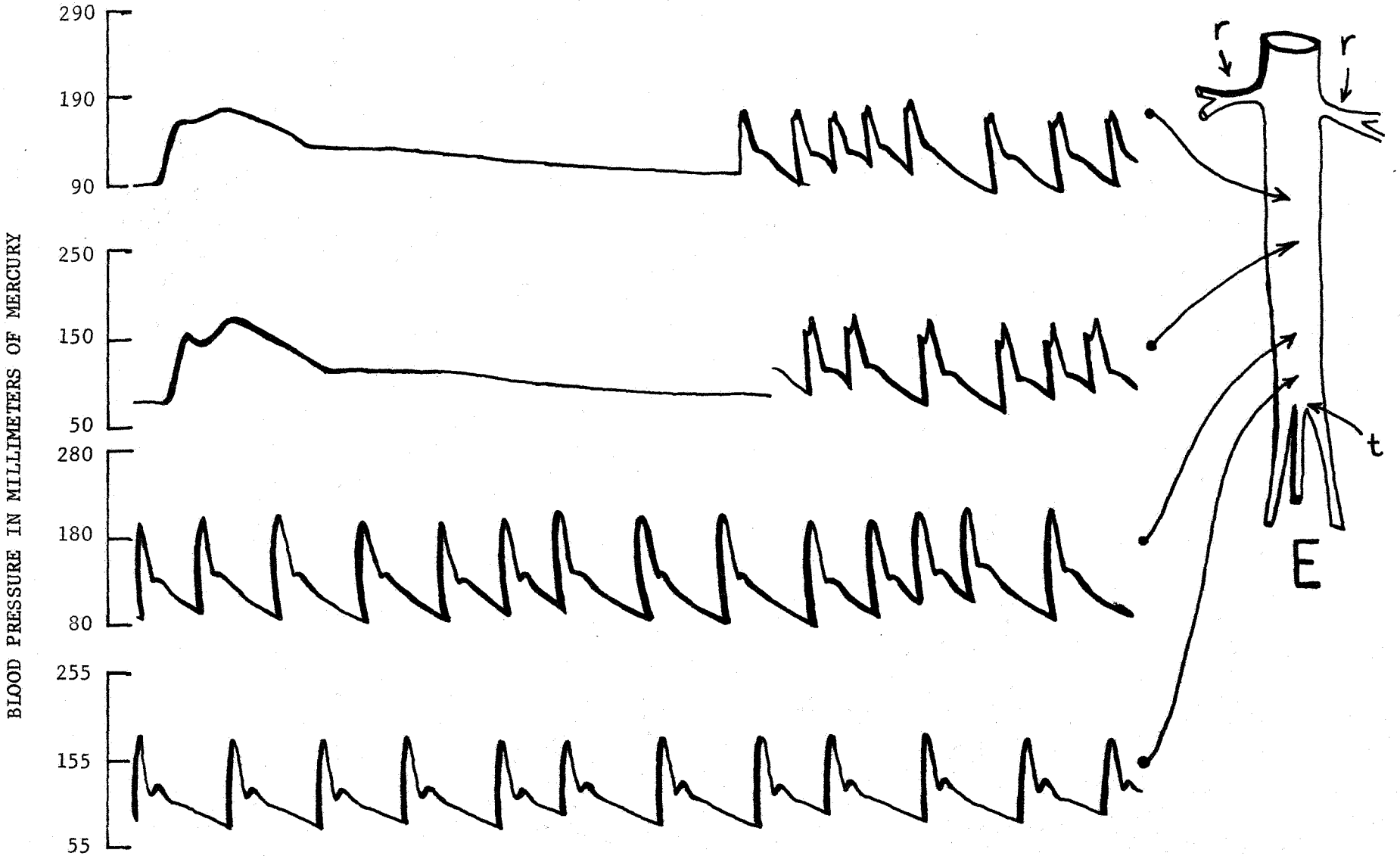

FIGURE # 9

Figure 9. Contours of pressure waves at different levels in the abdominal aorta.

Table I Abdominal Aortic Blood Pressures

DOG	Systolic Pressure	SD	Number of Observations	Diastolic Pressure	SD	Number of Observations	Pulse Pressure	Weight in kg
1. Russ(Mongrel)	138.0	9.2	200	81.0	12.1	200	57	20
2. Neron(Mongrel)	139.0	6.1	264	67.0	11.3	264	72	14
3. Carlyle(Mongrel)	140.0	10.4	140	77.0	10.0	140	63	20
4. Shag(Mongrel)	153.0	10.9	126	99.0	9.5	126	54	19
5. Pepper(Mongrel)	157.0	24.6	84	87.0	17.7	84	70	22
6. Troubles(Mongrel)	157.0	4.2	143	93.0	10.3	143	64	20
7. Foxy(Mongrel)	158.0	5.7	200	82.0	12.1	200	76	9
8. Nutmeg(Mongrel)	163.0	8.4	101	85.0	6.8	101	78	14
9. Fancy(Mongrel)	163.0	7.1	328	108.0	14.0	328	55	17
10. Watt(Beagle)	165.0	4.9	264	83.0	10.7	264	82	10
11. Aggie(Beagle)	166.0	6.8	100	83.0	10.9	100	83	6
12. Fats(Dalmatian)	167.0	10.5	167	89.0	11.1	167	78	25
13. Rover(Mongrel)	168.0	12.4	84	93.0	10.3	84	75	10
14. Gunter(Mongrel)	168.0	14.0	70	77.0	10.0	70	91	11
15. Ira(Mongrel)	169.0	15.7	224	83.0	12.2	224	86	14
16. Jeff(Beagle)	170.0	15.4	505	88.0	10.0	505	82	10
17. Floyd(Terrier)	171.0	7.2	222	86.0	10.9	222	85	13
18. Sigmund(Mongrel)	171.0	10.3	241	99.0	8.6	241	72	23
19. Siesta(Mongrel)	172.0	11.6	42	95.0	7.2	42	77	24
20. Spotty(Dalmatian)	172.0	14.8	506	108.0	13.7	506	64	19
21. Nestles(Mongrel)	172.0	21.8	112	86.0	13.4	112	86	22
22. Vicky(Beagle)	173.0	15.1	281	90.0	16.1	281	83	11
23. Hobo(Mongrel)	173.0	5.1	20	106.0	7.9	20	67	19
24. Rocky(Terrier)	173.0	39.1	180	90.0	36.2	180	83	13
25. Clove(Mongrel)	176.0	13.2	14	111.0	9.1	14	65	32
26. Shrimp(Beagle)	181.0	19.5	378	104.0	24.6	378	77	10
27. Durham(Mongrel)	184.0	13.9	140	94.0	11.9	140	90	16
28. Annie(Beagle)	187.0	7.4	100	95.0	10.1	100	92	10
29. Sirius(Spaniel)	192.0	11.9	314	77.0	12.0	314	115	11
30. Georgie(Mongrel)	199.0	11.6	145	114.0	11.8	145	85	14
Total $\bar{X}$	167.9		5,695	$\bar{X}$ 91.0		5,695		

SD = Standard Deviation

$\bar{X}$ = Average systolic or diastolic pressure

Table II Thoracic Aortic Blood Pressures

DOG	Systolic Pressure	SD	Number of Observations	Diastolic Pressure	SD	Number of Observations	Pulse Pressure	Weight in kg
1. Marie (Doberman)	121.0	8.9	80	78.0	11.4	80	43	21
2. Shrimp (Beagle)	144.0	7.9	109	91.0	10.0	109	53	10
3. Hobo (Mongrel)	147.0	6.6	20	109.0	8.5	20	38	19
4. Boush (Beagle)	148.0	14.2	400	89.0	12.2	400	59	11
5. Jeff (Beagle)	148.0	8.7	200	111.0	11.9	200	37	10
6. Sigmund (Mongrel)	155.0	9.4	498	98.0	8.2	498	57	23
7. Sirius (Spaniel)	158.0	7.2	139	86.0	8.8	139	72	11
8. Limon (Mongrel)	159.0	7.9	52	87.0	13.6	52	72	9
9. Blacky (Mongrel)	182.0	7.7	18	147.0	5.9	18	35	18
10. Amy (Beagle)	200.0	8.8	100	139.0	9.9	100	61	5
Total $\bar{X}$	156.2		1,616	$\bar{X}$ 103.5		1,616		

SD = Standard Deviation

$\bar{X}$ = Average systolic or diastolic pressure

CHRONIC PAINLESS RECORDING OF INTRA-ARTERIAL BLOOD PRESSURE IN UNANESTHETIZED DOGS.

The chronic recording of painless intra-arterial blood pressure in dogs was one of the most difficult problems in physiology until very recently. Most measurements of intra-arterial blood pressure required highly trained docile dogs in which the femoral artery was punctured with a cannula or needle (Pavlov, 1879; Kolls and Cash, 1923; Hamilton, Brewer and Brotman, 1934). This technique is limited in its use because continuous puncture can injure the artery and can be painful. The auscultatory method (Allen, 1923; Wilhelm, Waldman and McGuire, 1931), which avoids the injury to the artery, usually causes external inhibition in the animal during inflation of occlusion cuffs. Reliable recordings of indirect blood pressure can be taken only if the animals have been familiarized with the inflation of the occlusion cuff. Otherwise, the emotional disturbance will be superimposed on the recording of indirect pressure (Dykman and Gantt, 1960). Furthermore, the auscultatory method cannot be used for beat-by-beat measurements of blood pressure.

The solution of this problem has been possible due to scientific and technological progress: 1) advances in the development of stable and potent anticoagulants; 2) technological advances in the manufacturing of nontoxic plastics which neither react actively with biological tissues nor kink or crack easily; 3) advances in electronic instrumentation such as the development of sensitive strain-gage pressure transducers not available a few years ago; 4) last, but not least, advances in the technology of chronic implantation with the innovation of techniques for the proper fixation of blood-pressure catheters to arteries using anchoring rings built in or glued to the plastic tubing (Gaertner, 1964).

Methods

Blood pressures were recorded inside a soundproof room with an attenuation of 81 db. The room temperature was controlled to 74° F. Techniques for the implantation of blood-pressure catheters have been described elsewhere (Perez-Cruet, Plumlee and Newton, 1966). The blood pressure was measured with a Statham P23De strain-gage pressure transducer. Calibration of the strain-gage was performed with a mercury manometer and readings were made in millimeters of mercury (mm Hg). Most measurements reported in this study were taken at the beginning of an experimental session, 10 to 15 minutes after the animals were brought into the soundproof room. The measurements of blood pressure were taken from ten consecutive pressure pulses for each experimental day. Direct systolic and diastolic blood pressures were measured by taking the reading at the peak of the pressure pulse as the systolic reading and the lowest end of the downstroke of the pressure pulse as the diastolic reading. Blood pressures have been determined for periods ranging from 8 to 12 months with catheters implanted through the femoral artery into the abdominal aorta.

Results and Discussion

On reviewing the literature, we have not found any reports showing chronic recordings of blood pressure using chronically implanted intra-arterial catheters. Figure 1 and 2 illustrate intra-arterial blood pressure measurements from the lower third of the aorta of dogs Neron and Sirius, for a perod of 8 to 12 months. The blood pressure varied between 140 to 204 systolic and 57 to 123 diastolic for Sirius, and between 135 to 202 systolic and 62 to 119 diastolic for Neron. The two dogs in this report had been exposed to chronic hypothalamic stimulation and had shown transient conditional hypertension to

an auditory stimulus but did not develop permanent hypertension. It was found that blood pressures were higher for the winter months in both animals but the mechanism for this response is not well understood. A definite increase in basal systolic blood pressures was observed after another series of experiments in which hypothalamic self-stimulation was employed, as indicated by a heavy arrow in Figure 2. The variability in day-by-day recording of blood pressures has been observed by other investigators using indirect methods.

Figure 3 shows transient effects produced by hypothalamic self-stimulation. The transient neurogenic hypertension produced by hypothalamic electrical self-stimulation adapted very quickly after the electrical stimulation had been turned off in spite of the fact that the dog continued to press the lever very actively. This is one example of what we call schizokinesis (Gantt, 1953, 1960), namely a dysfunction between the specific motor behavior and the autonomic nervous system (blood pressure) changes. Schizokinesis in this example is manifest as an adaptation of the blood pressure during non-stimulating lever-pressing behavior, that is, the blood pressure is returned to a baseline level while the animal's lever-pressing behavior (specific motor behavior) is activated. In this dog also, a transient conditional hypertension to a conditional auditory stimulus previously paired with hypothalamic stimulation for a period of one year could not be totally extinguished after 3,000 continuous presentations of the conditional auditory stimulus as shown in Figure 4.

Our curves of chronic recording of direct blood pressure in unanesthetized animals are similar to the chronic recording of indirect blood pressure curves reported by Ferris and Hynes (1930). The direct blood pressure readings in our study were higher than those reported by Ferris and Hynes, because they were recorded from the lower third of the aorta where peripheral pressures are usually higher than indirect pressures. In normal dogs, the range of peripheral aortic pressures varies between 138/67 and 199/114 mm Hg.

The success of recording direct intra-arterial blood pressures over several months opens the field for the development of new objective techniques for studying the role which emotional factors play in the development of experimental hypertension of psychogenic origin.

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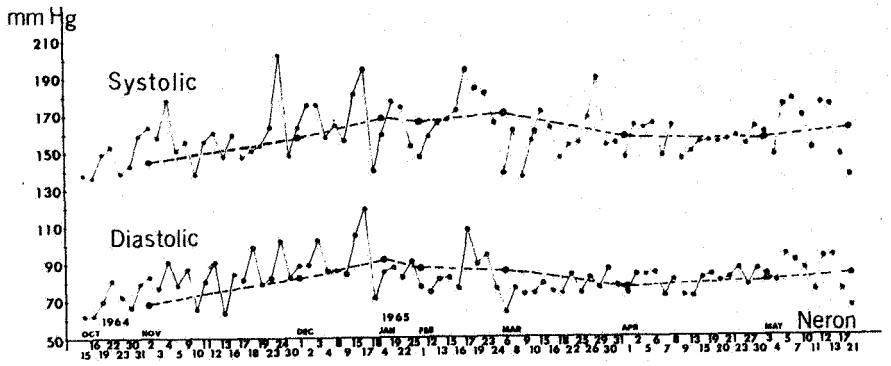

Fig. 1. Chronic direct blood pressures recorded for 8 months. Broken line indicates the average blood pressure per month.

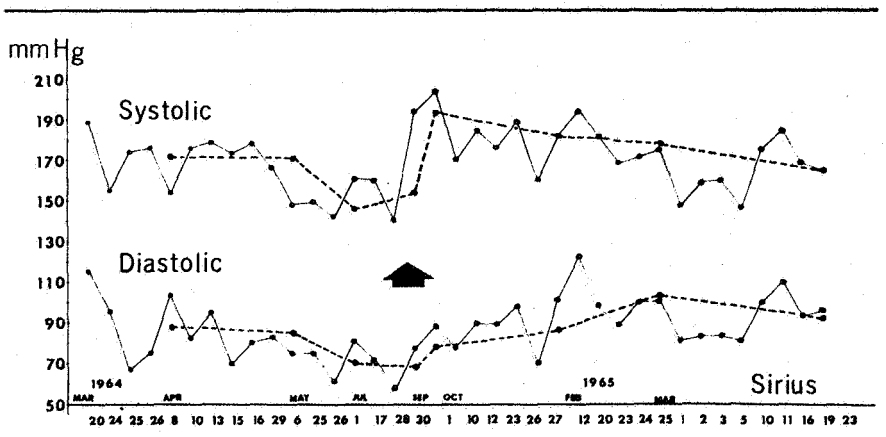

Fig. 2. Chronic direct blood pressures recorded for 12 months. Heavy arrow indicates the effect of self-stimulation experiments on basal blood pressure. Broken line is average blood pressure per month.

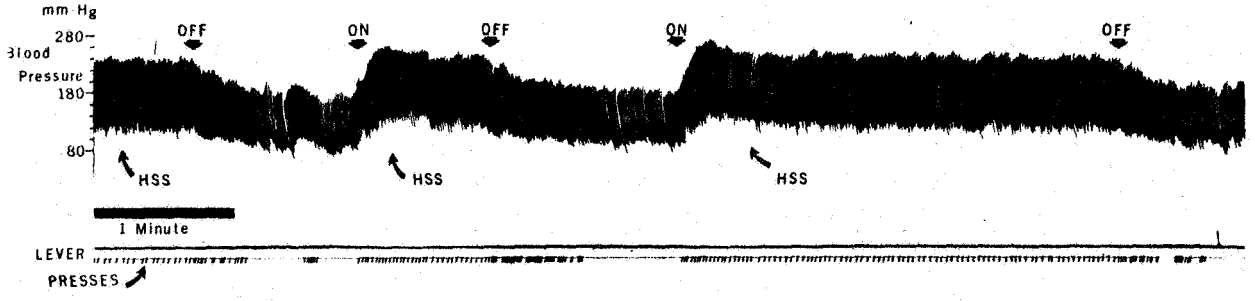

Fig. 3. Transient neurogenic hypertension produced by hypothalamic self-stimulation. ON indicates period in which lever presses can stimulate the hypothalamus electrically. OFF indicates period in which lever presses do not stimulate the hypothalamus electrically. Current intensity was 1 milli-ampere. HSS = hypothalamic self-stimulation.

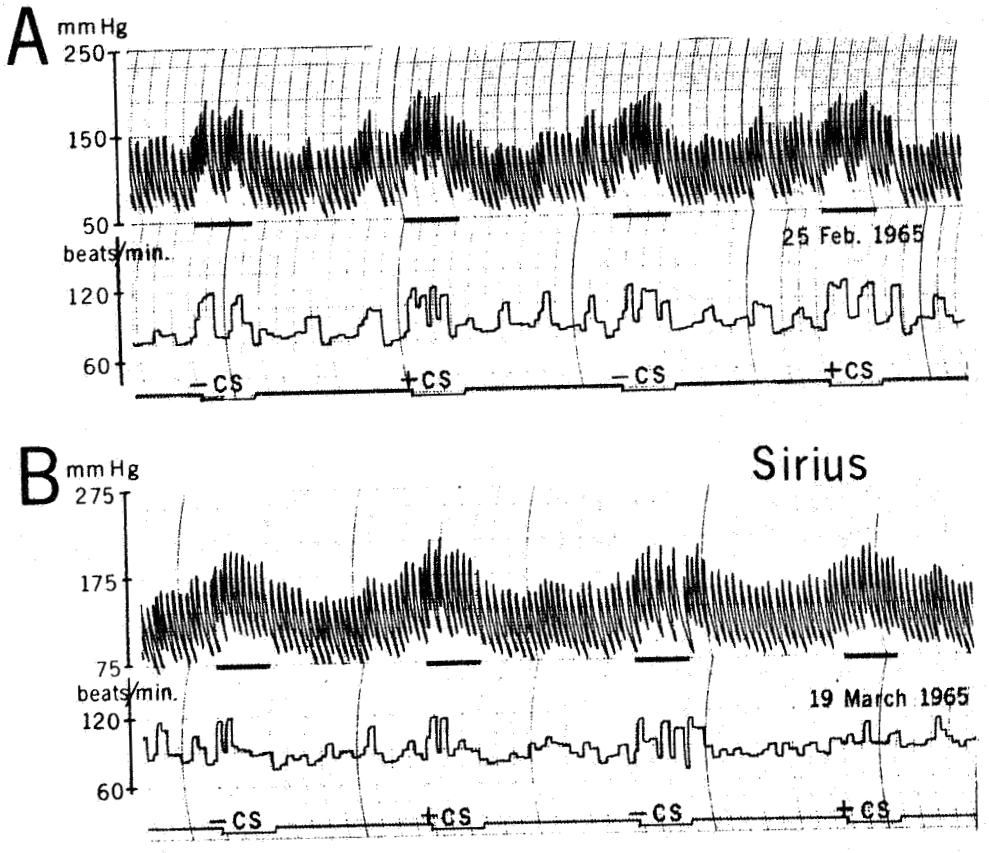

Fig. 4. Blood pressure and instantaneous heart rate responses to an auditory conditional stimulus during extinction. A, cardiovascular conditional responses at the beginning of extinction. B, after 3,000 extinction repetitions of the conditional stimuli. +CS excitatory tone (T 256 or T800). -CS inhibitory tone (T 512 or T 1600). Paper speed was 5 mm/sec.

BLOOD-PRESSURE AND HEART-RATE CHANGES IN DOGS DURING HYPOTHALAMIC SELF-STIMULATION·

Recently several reports have been published concerning heart-rate (HR) changes during septal and hypothalamic self-stimulation in rats (Malmo, 1961; Perez-Cruet, Black, & Brady, 1963). Septal self-stimulation usually produces a deceleration of HR whereas hypothalamic self-stimulation (HSS) usually produces acceleration. The magnitude and direction of HR changes depend on the location of the electrodes within these areas. Malmo (1964) found that stimulation of the lateral septal area produces deceleration of HR but more medial placements produce an initial acceleration followed by a deceleration.

Although the above studies have shown definite cardiovascular changes during self-stimulation in rats, there was no information available to us that these same changes occur in other species. Furthermore, most studies concerned with the study of cardiovascular components of self-stimulation have measured only HR. It is the purpose of this investigation to study blood pressure (BP) and HR during HSS in dogs and to determine to what extent these cardiovascular components are important in supporting self-stimulation.

Method

Subjects: The Ss were four healthy, 3-5 yr. old, male dogs (two terriers, a beagle, and a spaniel) weighing 20-24 lb.

Electrodes and Stimulus Parameters: One or two enameled platinum bipolar electrodes were implanted stereotaxically in the hypothalamuc using a dog atlas (Lim, Liu, & Moffitt, 1960). Electrode assembly and implantation techniques as described elsewhere (Sheatz, 1961; Valenstein, Hodos, & Stein, 1961) were modified for Ss.

The brain stimulus consisted of a .5 sec. train of biphasic rectangular pulses presented at a frequency of 100/sec. Pulse duration and delay between positive and negative pulses were .2 msec. The current intensities, measured during the steady-state plateau, varied between .5 and 1.8 ma. (approximately 5-18 v.). Electrode resistances were approximately 10,000 ohms.

Apparatus: A commercial (Industrial Acoustics Company, New York) soundproof room, Model 1200, 7 x 7 x 7 ft., with a sound attenuation of 81 db. was used. Time periods during which lever presses would produce electrical hypothalamic stimulation were programed with transistorized equipment. The HSS responses were recorded in Gerbrand's cumulative recorders and at the same time counted in electromechanical counters. A Lehigh-Valley Electronics lever, Model 1380, was used to activate stimulation to the hypothalamus during lever presses. The brain stimulation unit consisted of Tektronix components (two, Type 161, pulse generators; two, Type 162, wave-form generators; a monitoring oscilloscope, Type 360; and a transistorized isolation unit). A Gilford cardiotachometer was employed to measure instantaneous beat-to-beat HR. A Statham transducer P23De was used to measure arterial BP in millimeters of mercury (mm Hg), beat-by-beat, from a catheter in the femoral artery. A strain-gauge transducer was used to record respiration. An 8-channel Offner polygraph was employed for recording physiological variables.

Procedure: Training and experimental. The Ss were trained for several weeks after the operation. In one S the training for HSS was done after 1 yr. of implantation.

The S was harnessed lightly on a platform directly in front of which a Lehigh lever had been placed, so that S could press the lever with the nose or foreleg. Silver electrodes were fastened with masking tape to S's side over the thoracic region and also on the left hind leg. A strain-gauge transducer respirometer was attached around the chest. Finally, S was connected to the brain stimulator unit through the cranial connectors. The soundproof room was closed and S was observed through a one-way mirror or through a closed television circuit during the experiments. The initial shaping of HSS usually took 15-45 min. in the first experimental session. One or 2 days after this initial training the experimental sessions were brought under stimulus control, which consisted of 5-min. periods of HSS (light "on") alternating with 5-min. periods of nonstimulation (light "off") when pressing the lever produced no HSS. Lever responses and electrocardiograms (EKG) were recorded separately for each on and off interval.

For several experimental days the HR was recorded during HSS. After this initial training Ss were anesthetized in order to implant chronically a polyvinyl catheter (Suprenant Manufacturing Company, Clinton, Massachusetts, Surco-1 clear 0.038 x 0.070 in.) in the femoral artery; the other end of the catheter was brought to the neck of S and occluded with a stainless-steel plug. The catheters were usually flushed everyday with 1 cc of heparin sodium, 1,000 USP units/cc, (1 unit = .01 mg.) Two days after surgery the BP and HR were determined before and during self-stimulation.

At the completion of the experiments three Ss were sacrificed and perfused. One S, Sirius, was spared. Stereotaxic coordinates for Sirius were: (R) rostral to the interaural line, 19 mm.; (L) lateral to the midline, 2 mm.; (V) vertical above the horizontal zero plane, 5 mm. Frozen sections cut at 50 and stained with Nissl stain provided histological confirmation of the electrode placements.

Measures: Stable patterns of HSS were established by the time measurements began. The HR was determined by counting the number of R waves in consecutive 10-sec. intervals of time and then converting these numbers to beats per minute (bpm) by multiplying by 6. Instantaneous beat-to-beat HR was obtained simultaneously with a Gilford cardiotachometer. Systolic BP was analyzed by measuring the highest systolic BP was measured in a similar manner. Counts of 20-sec. intervals were used; otherwise a considerable amount of labor would have been employed by counting BP for each individual heart beat.

Means, SD, and distribution patterns were obtained for each individual S before and during HSS periods. The statistical evaluation of the data was based on t tests for paired observations (correlated means). Mean BP values for all 20-sec. intervals during nonstimulation and HSS periods were compared. The same statistical analysis was applied to the HR measurements.

Lever presses were recorded in counters and in Gerbrands' cumulative recorders. The lever presses were also counted directly from the Offner records during 10-sec. intervals.

Respiration was evaluated by measuring and observing the fluctuations in the respiratory tracings.

Drug Studies: Several drugs were employed to test various psychophysiological mechanisms involved in our studies. Curare (d-tubocurarine chloride), which produces muscular paralysis at the peripheral nerve endings, was used in doses of 2 units/kg (2 units = .3 mg.) in two Ss to determine the role of muscular movements in physiological responses associated with HSS. Adrenergic blocking agents, (intravenous doses of 5 mg/kg of dibenzyline and intravenous

doses of 3 mg/kg of dichloroisoproterenol) were used in two Ss to determine the effect of blocking of the sympathetic system on the physiological responses.

Results

Each of the four Ss showed an increase in mean systolic and diastolic arterial pressures during HSS as shown in Table 1. All Ss showed normal BP levels during nonstimulation periods. Very high BP levels during HSS were developed by S_3 and S_4. Significant increases in HR during HSS were shown by S_1, S_3, and S_4 (Table 2), but S_2's changes in HR during HSS were not significant. All t-test comparisons between mean BP values during nonstimulation and HSS periods were highly significant ($p < .001$).

Figure 1 illustrates selected tracings from the Offner in two Ss. Tracing A shows a response delay of 18 sec. between the L-ON and the beginning of HSS. Note that prior to HSS and after L-ON there was a decrease in BP from 166/64 to 140/54 mm. Hg; the respiratory tracing became more irregular; and the average HR increased from 90 to about 108 bpm. The average BP during the nonstimulation period was about 150/60 mm. Hg. During HSS, the BP increased to 250/150 mm. Hg; the respiratory rate increased from 18 cycles/min to about 54 cycles/min and the respiratory recording became irregular due to artifacts of movements; the HR increased from 88 bpm (prestimulus level) to 178 bpm during HSS. The average lever-pressing rate was approximately 96 lever presses/min. In tracing B too, a delay between L-ON and the heavy arrow is observed. Note that during the delayed interval this S showed no cardiovascular changes. The average HR during the nonstimulation period was 72 bpm and it increased to 91 bpm during the HSS period, the respiratory rate increased from 15 cycles/min to 30 cycles/ min. The respiratory tracing was not affected by movements. The BP increased from 180/76 mm. Hg during nonstimulation to 266/124 mm. Hg during HSS. The lever pressing rate was 48 lever presses/min.

All Ss showed good HSS rates. Lever-pressing rates for S_1, S_2, S_3, and S_4 were 0.6, 1.8, 0.3 and 0.5 lever presses/min (averages of 350 intervals of 10 sec.) during the nonstimulation period and 92.4, 105.0, 53.0, and 87.6 lever presses/min (average of 350 intervals of 10 sec.) during HSS, respectively.

In order to rule out the possibility that the cardiovascular changes during HSS were not due to the movements associated with the lever pressings, simulated HSS rates were given manually while two Ss were fully paralyzed with curare and ventilated artifically. Figure 2 is a selected record that shows the effect of curare on cardiovascular functions during manual hypothalamic stimulation. Under curare (.3 mg/kg), the HR response was obliterated (control, nonstimulation = 90 bpm, HSS = 202 bpm; under curare, nonstimulation = 92 bpm, manual stimulation = 68 bpm) but the BP response was not changed (control, nonstimulation = 150/80 mm. Hg, HSS = 250/150 mm. Hg; under curare, nonstimulation = 155/85 mm Hg, manual stimulation = 250/150 mm. Hg).

The blocking effects of curare on the HR response during manual stimulation were observed several times within an experimental session. In the curarized Ss, no muscular contractions were visible during manual stimulation but as soon as the effect of the drug began to disappear, manual stimulation produced gross muscular twitches which were accompanied by an increase in HR. Table 3 summarizes the results with curare in two Ss. The differences between mean HR and BP values during nonstimulation and HSS periods before curare were statistically significant. In the same Ss, the differences between mean HR and BP during nonstimulation and manual stimulation periods under curare were less significant for HR than for BP. Although HR changes were significant

for S_4, under curare, the mean difference in HR before curare was 77.1 bpm whereas under curare the mean difference was 11.5 bpm.

In order to determine to what extent these cardiovascular components are important in supporting HSS, drug studies were performed using adrenergic blocking agents dibenzyline and dichloroisoproterenol (DCI). The use of these drugs did not reduce significantly the HSS rate but they did affect the cardiovascular responses which occur during HSS as shown in Figure 3. The increase in BP during HSS was completely blocked by dibenzyline (5 mg/kg). The BP during the nonstimulation period was 100/60 mm. Hg and during the HSS period remained 98/60 mm. Hg, although the HR increased from 156 bpm to 288 bpm during HSS. When DCI was injected in doses of 3 mg/kg after dibenzyline, the accentuated HR response was reduced from 288 bpm to 224 bpm as illustrated in Part B of Figure 3.

The effects of dibenzyline were analyzed in two Ss. These results are summarized in Table 3. All differences between mean BP and HR values during nonstimulation and HSS periods before dibenzyline were statistically very significant. In the same Ss, the differences between the mean HR and BP during nonstimulation and HSS periods under dibenzyline were less significant for BP than for HR. At this point we want to emphasize that HSS continued even though the BP response during HSS had been blocked. Under dibenzyline and DCI the HR differences were less significant than with dibenzyline alone for S_3 (-.6 bpm, $t = .2$, $df = 55$, $p < .9$) and S_4 (15.9 bpm, $t = 7.5$, $df = 284$, $p < .001$). All BP responses became more significant during DCI but the mean BP differences were still much less than those observed before the injection of dibenzyline: S_3 (systolic BP diff. = 22.9 mm. Hg, diastolic BP diff. = 14 mm. Hg) and S_4 (systolic BP diff. = 17.9 mm. Hg, diastolic BP diff. = 12.2 mm. Hg).

Histological verification of the rewarding placements revealed the tips of the electrodes to be within the right mammillothalamic tract and the dorsal part of the medial mammillary body in S_1 (Rocky), within the Fields of Forel immediately dorsal to the zona incerta in (Floyd), and in the poseterior hypothalamic area immediately dorsal, and adjacent to, the anterior mammillary nucleus in S_4 (Shrimp). Figure 4 shows the actual histological sections where the electrode tips were located (see arrows).

Discussion

The study reveals definite changes in the cardiovascular system of dogs during HSS similar to those reported in rats by Perez-Cruet et al. (1963). These changes are mediated via the sympathetic system from the hypothalamus as reported by Hess (1957) and Beattie, Brow, and Long (1930). The results clearly show an increase in BP and HR during self-stimulation from placements in the posterior hypothalamuc and mammillary bodies.

The results with curare and the adrenergic blocking agents have shed some preliminary information upon the mechanisms of cardiovascular changes during HSS. Under curare, the BP response to manual stimulation was not affected whereas the HR response was blocked. These findings clearly indicate that the BP response does not depend on muscular movement or on the HR response during stimulation. Blockage of the HR response, under curare, suggests that this response during HSS is physiologically bound to muscular movement. However, there are other possible mechanisms by which the HR response can be modified. For example, curare can affect the autonomic ganglia (Langley, 1918)

and interfere with the neural mediation of the HR response. Furthermore, there is recent evidence which indicate that some central HR responses can be independent of movement (Black, Carlson, & Solomon, 1962; Newton & Gantt, 1960; Perez-Cruet & Gantt, 1959).

The results with curare also show that the increase in BP during HSS does not depend on respiration, for the respiratory rate was constant during artificial respiration. The possibility that the HR response is secondary to a respiratory change cannot be ruled out. However, Malmo (1963) has shown that the HR response to septal self-stimulation is independent of respiration. Furthermore, two studies from our laboratory have shown that HR changes can be independent of respiratory rate (Perez-Cruet & Gantt, 1961; Perez-Cruet, Newton, & Gantt, 1965).

The results under dibenzyline show that the BP response was completely blocked, the HR response was accentuated, and the self-stimulation behavior was not affected. When DCI was injected after dibenzyline, the HR response was diminished and the BP response increased slightly during HSS. These results suggest that the BP response is not causally related to self-stimulation because, if it were, its blockage by dibenzyline would have disrupted self-stimulation. On the other hand, these results indicate an interrelationship between BP and HR because the HR response was accentuated when the BP response was blocked. The accentuation of the HR response was probably produced by stimulating other cardiovascular reflexes when the BP was lowered by dibenzyline or by homeostatic mechanisms. Support for the last interpretation, of homeostatic balance between BP and HR responses, is given by the results from the injection of DCI following dibenzyline. As soon as the accentuated HR response was decreased in magnitude, a BP response reappeared. The reciprocal relationships between BP and HR are not clearly understood; e.g., a fall in BP can produce an increase in HR even after the heart has been completely denervated (Best & Taylor, 1961, p. 305).

Stark, Boyd, and Fuller (1964) have recently shown that neurohormones are of major importance in maintaining HSS in dogs. Their work suggests that more than one neurohormone may be involved in maintaining HSS. Our results indicate that blocking the adrenergic system with dibenzyline does not block the HSS and also suggest that probably other autonomic responses and neurohormonal mechanisms may be involved in maintaining HSS.

It appears from all previous studies on self-stimulation in rats, and now in dogs, that, whenever there is self-stimulation, concomitant autonomic changes can be monitored. This fact suggests that self-stimulation behavior is probably a central phenomenon that involves the autonomic nervous system.

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TABLE 1

MEAN SYSTOLIC AND DIASTOLIC BLOOD PRESSURES (IN MM. HG) BEFORE AND DURING HSS IN FOUR *Ss*

S	*N*[a]	Systolic				Diastolic			
		Nonstimulation		Self-stimulation		Nonstimulation		Self-stimulation	
		M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Rocky	281	172.0	39.1	215.5	33.7**	90.0	36.2	126.3	37.2
Floyd	368	170.0	7.2	190.3	12.0**	86.0	10.9	101.9	16.9
Sirius	596	191.5	11.9	258.5	17.0**	76.6	12.2	114.4	16.1
Shrimp	602	180.5	19.5	249.3	18.9**	104.1	24.6	147.1	17.5

[a] *N* indicates number of individual 20-sec. interval measurements.
** $p < .001$.

TABLE 2

AVERAGE HEART-RATE CHANGES (IN BPM) BEFORE AND DURING HYPOTHALAMIC SELF-STIMULATION IN FOUR *Ss*

S	*N*[a]	Heart rate (in bpm)			
		Nonstimulation period		Self-stimulation period	
		M	*SD*	*M*	*SD*
Rocky	907	94.5	16.2	110.0**	12.3
Floyd	911	104.8	14.0	104.9	7.7
Sirius	1579	81.0	16.0	104.0**	19.2
Shrimp	1383	110.9	28.3	158.9**	25.2

[a] *N* indicates number of individual 10-sec. interval measurements.
** $p < .001$.

TABLE 3

EFFECT OF CURARE OR DIBENZYLINE ON HEART RATE AND BLOOD PRESSURE DURING MANUAL HYPOTHALAMIC SELF-STIMULATION

Experimental condition	Mean HR (in bpm)				Mean systolic BP				Mean diastolic BP			
	Nonstimulation	HSS	*t*	*df*	Nonstimulation	HSS	*t*	*df*	Nonstimulation	HSS	*t*	*df*
Rocky: Control	117.3	120.6	2.3*	140	228.3	256.1	13.6**	92	150.3	169.3	11.9**	92
Rocky: Curare	102.5	101.6	.8	268	228.3	255.9	13.7**	136	149.1	170.3	11.5**	136
Shrimp: Control	106.6	183.7	19.3**	176	162.5	247.4	26.0**	84	89.4	106.6	18.3**	84
Shrimp: Curare	81.7	93.2	3.5**	185	194.4	278.1	28.7**	78	100.5	146.2	19.2**	78
Sirius: Control	90.0	135.0	9.7**	64	175.4	267.1	16.4**	32	60.4	112.5	14.6**	32
Sirius: Dibenzyline	100.2	154.2	24.9**	64	167.8	172.3	1.2	32	80.9	96.7	6.1**	32
Shrimp: Control	111.0	150.6	24.9**	521	174.4	256.1	50.6**	280	91.5	144.3	33.0**	280
Shrimp: Dibenzyline	135.5	229.3	23.0**	383	124.1	119.1	−1.9	193	82.2	79.7	−1.3	193

* $p < .05$.
** $p < .001$.

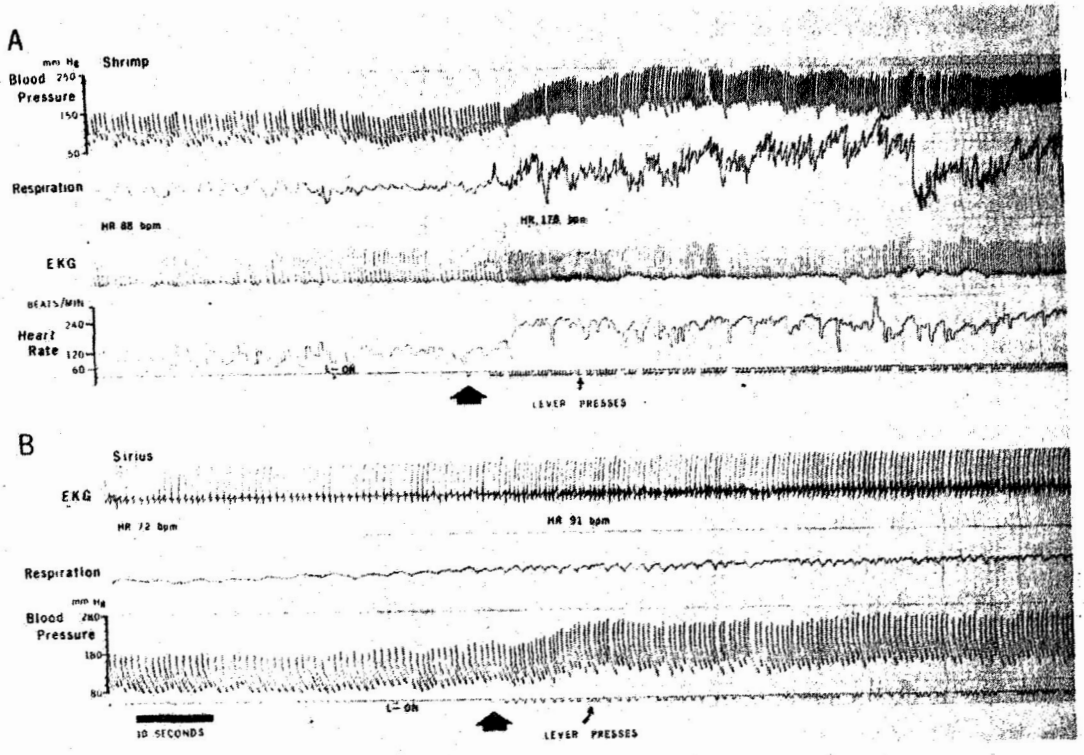

Fig. 1. Polygraph tracings showing changes in BP, respiration, and HR during HSS with current intensity of 1 ma. (L-ON indicates that light is on; heavy arrow indicates beginning of HSS. A, from top to bottom, shows systolic and diastolic BP, respiration, EKG, beat-by-beat HR, and lever presses. B, from top to bottom, shows EKG, respiration, BP, and lever presses.)

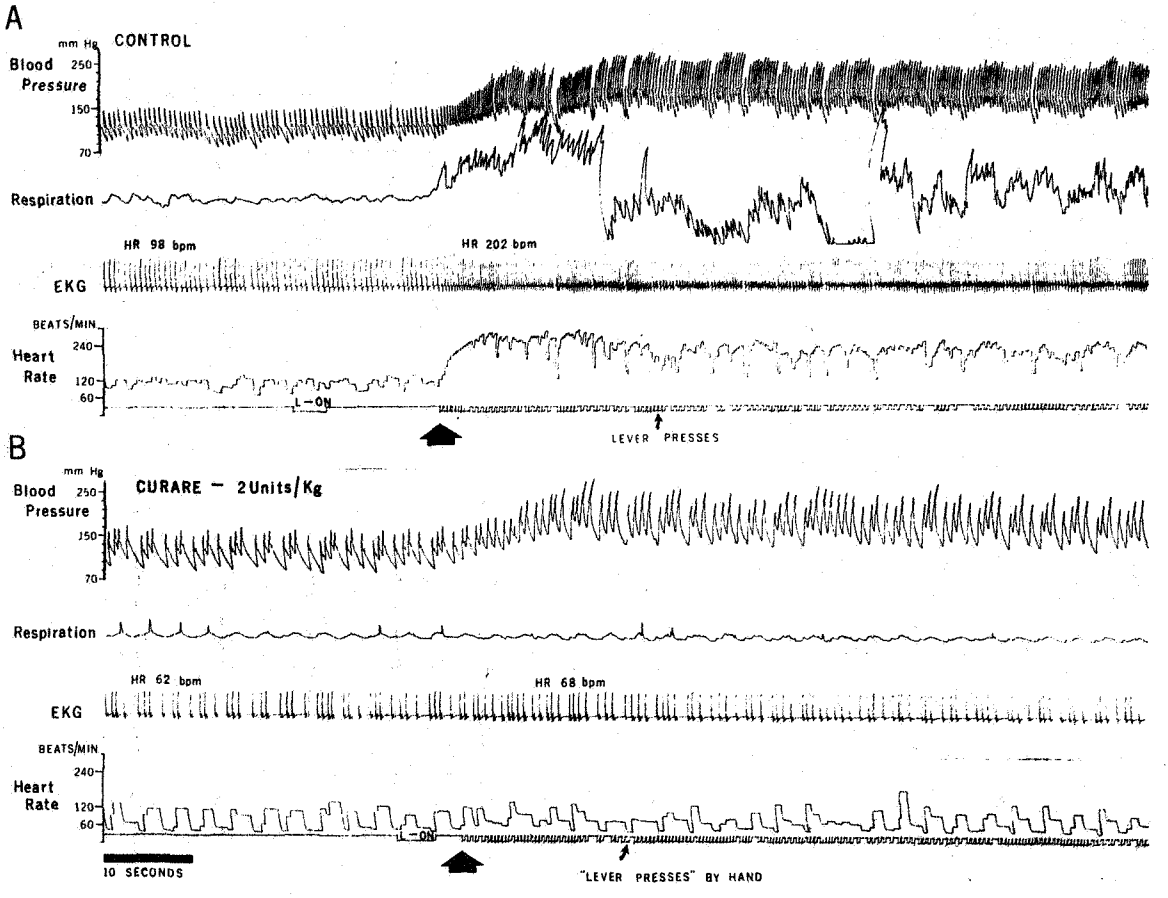

Fig. 2. Polygraph tracings showing changes in BP, respiration, and HR during HSS before (A) and after (B) the intravenous injection of .3 mg/kg of curare. (Heavy arrow in A indicates beginning of HSS; at B it indicates simulated "lever presses" by hand. Note that the BP response to manual hypothalamic stimulation is not blocked by curare.)

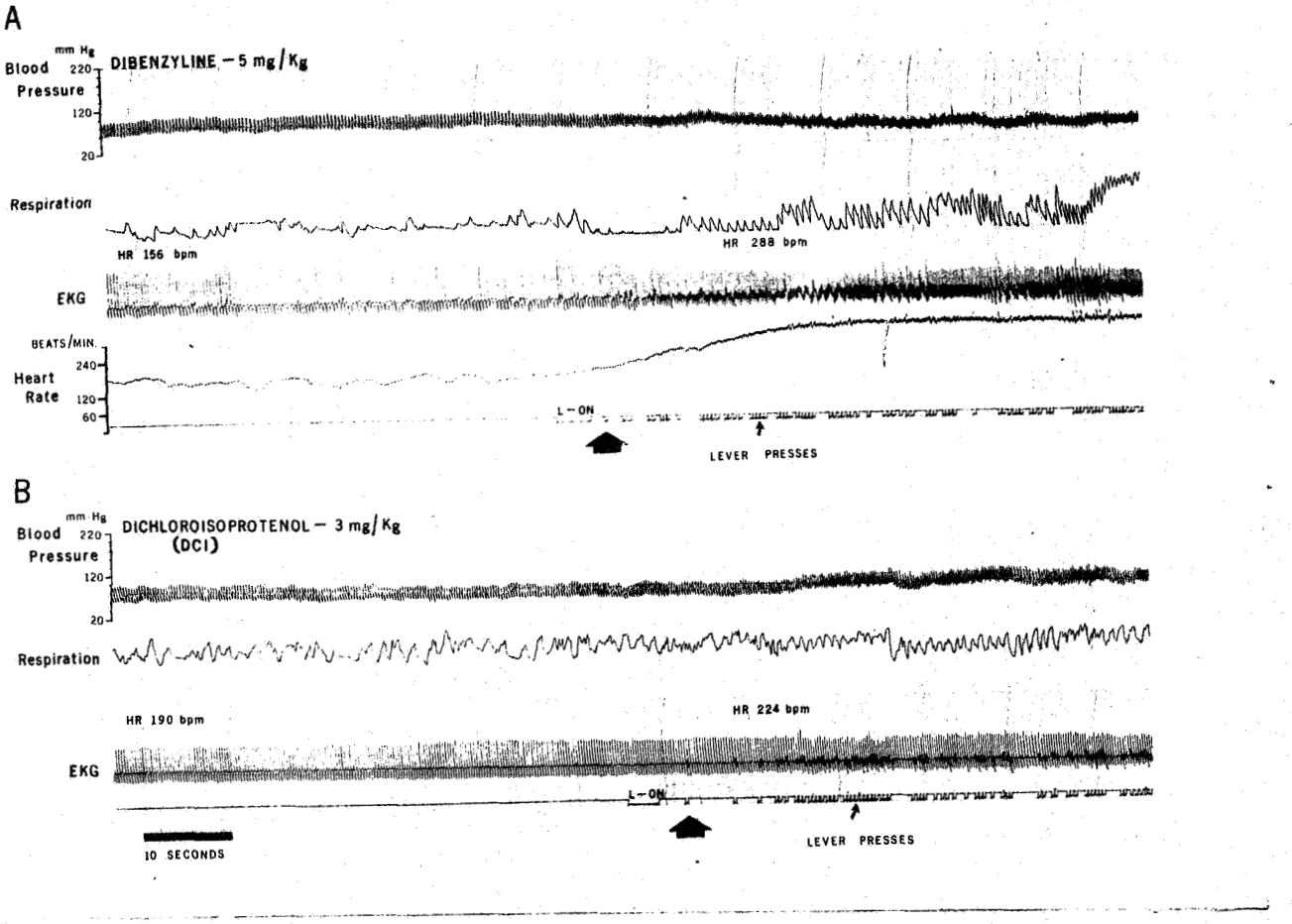

Fig. 3. Polygraph tracings showing changes in BP, respiration, and HR under adrenergic blocking agents, dibenzyline (A) and DCI (B).

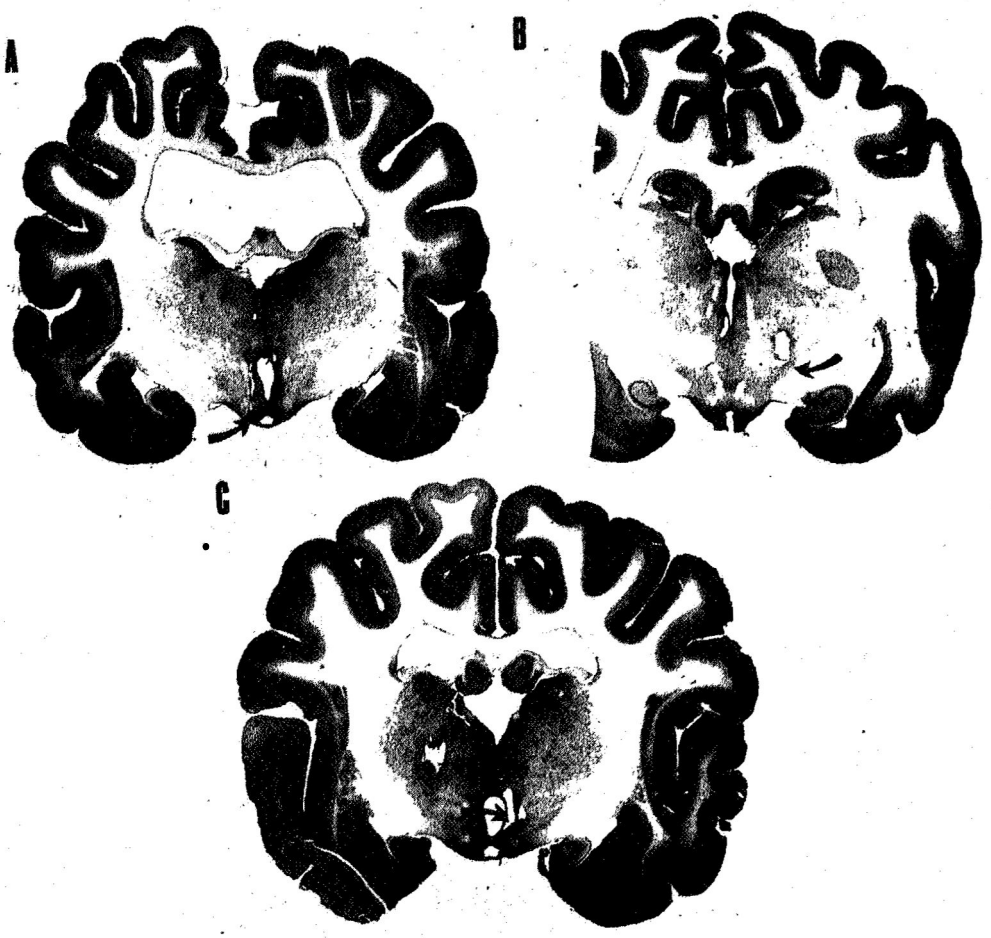

Fig. 4. Histological sections cut at 50u and stained with Nissl stain. (A, Rocky; B, Floyd; and C, Shrimp. Curved arrow point the location of the rewarding area.)

SUBCORTICALLY EVOKED CARDIOVASCULAR RESPONSES USING ELECTRICAL STIMULATION IN MONKEYS.

The purpose of this study is to determine the changes in heart rate and blood pressure which are produced by stimulation of anterior or posterior hypothalamus, putmen and septal areas. This work is an elaboration of previous work using electrical stimulation as an unconditional stimulus (US) and the effects of self-stimulation as an unconditional stimulus (US) and the effects of self-stimulation behavior on cardiovascular functions (Perez-Cruet, Brady and Black, 1963; Perez-Cruet, McIntire and Pliskoff, 1965). The study is also designed to determine the differences in cardiovascular responsivity from several subcortical areas.

Ten monkeys have been employed in this study and electrodes have been placed stereotaxically in the above areas. The monkeys have been restrained in Foringer chairs using equipment and stimulation parameters described elsewhere (Perez-Cruet, et al. 1965).

Preliminary results showed significant changes in cardiovascular functions, heart rate and blood pressure, in three monkeys during septal, anterior and posterior hypothalamic stimulation respectively.

Stimulation of the septal area usually produced a sudden drop in heart rate (about 30 to 40 bpm) followed by an acceleration of about 20 bpm from a baseline level. The blood pressure changes from stimulation of this area showed initially a slight diminution but no increase in pressure during the tachycardic phase of the biphasic response. These responses were elicited at levels of current corresponding to 50 μa and they became more accentuated at 1 ma.

Stimulation of the posterior hypothalamus showed consistently an increase in blood pressure (about 10 mm Hg.) in both systolic and diastolic pressures. The blood pressure change after stimulation usually persisted for 2 to 5 seconds after a single stimulation. In some experiments another blood pressure pattern response was observed in which an initial increase in blood pressure was followed by a decrease and then a subsequent increase was observed after stimulation at levels of 3 ma. This saddle-shaped blood pressure response has been observed previously in dogs from the lateral hypothalamus. The changes in heart rate produced by stimulation of the posterior hypothalamus consisted of an initial acceleration which lasted for 3 to 4 cardiac cycles followed by a deceleration. These effects were opposite to those seen during septal stimulation.

Stimulation of the anterior hypothalamus showed an initial increase in heart rate which lasted for 1 cardiac cycle (from 10 to 20 bpm) followed by a significant decrease in heart rate from 40 to 65 bpm. The heart rate usually remained below or returned to baseline during anterior hypothalamic stimulation. Blood pressure changes during stimulation of the anterior hypothalamus showed a very slight (about 2 to 5 mm Hg.) increase after stimulation. These effects from the anterior hypothalamus were obtained at current levels of 3 to 5 ma. The above responses from septal, anterior and posterior hypothalamus were duplicated at levels of stimulation from 100 μa to 1 ma, but if several stimulations lasting about $\frac{1}{2}$ second were presented in succession then the current levels required to produce the cardiovascular responses were lower (50 to 200 μa).

This data shows the different patterns of cardiovascular responses from the septum, anterior and posterior hypothalamus. None of the monkeys that showed these cardiovascular changes developed behavioral disturbances or seizures. An arousal phenomenom was observed during stimulation. In none of these monkeys could self-stimulation be elicited in spite of the fact that the animals were showing cardiovascular changes during stimulation.

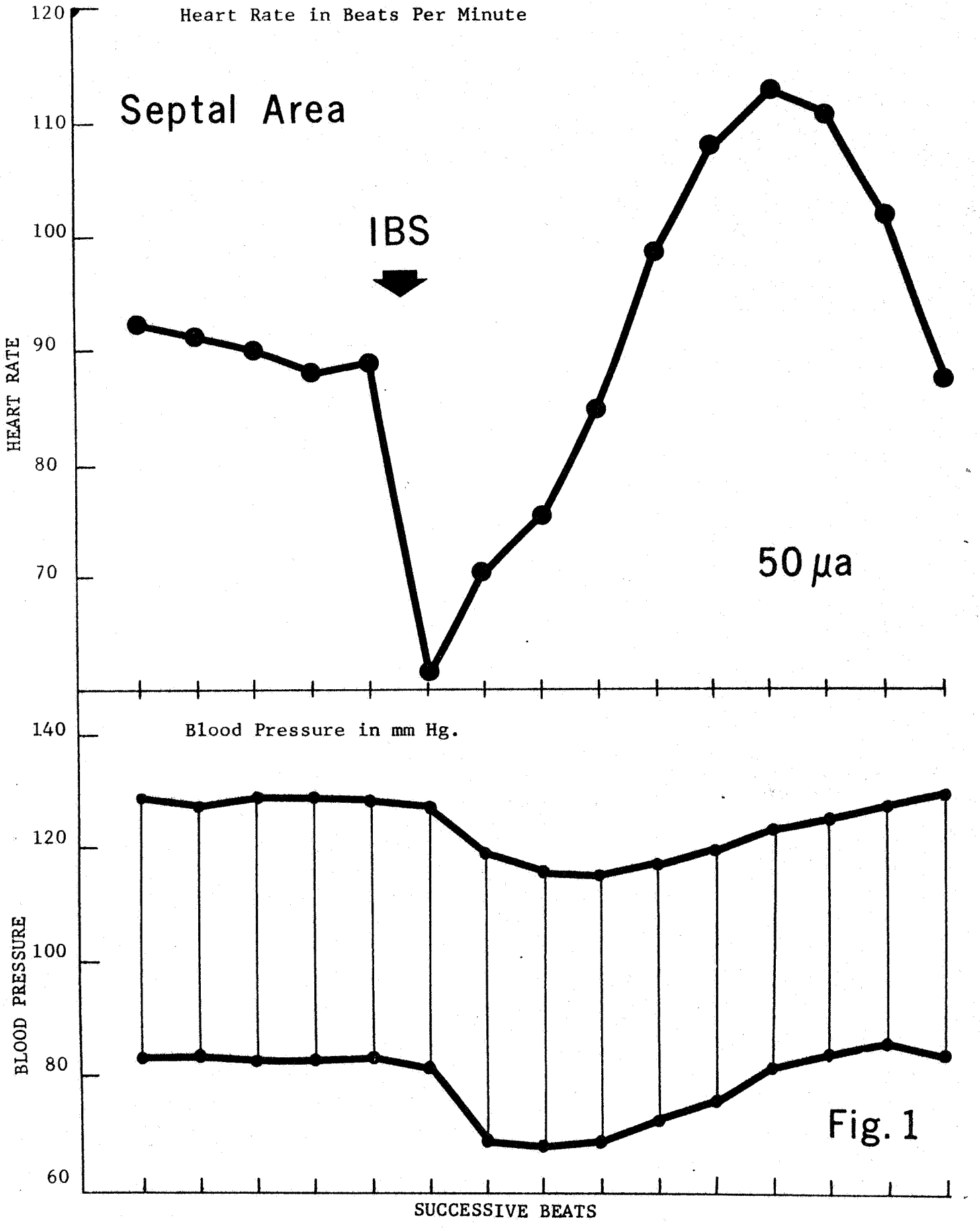
Heart Rate in Beats Per Minute
Septal Area
IBS
50 μa
HEART RATE
120
110
100
90
80
70
Blood Pressure in mm Hg.
BLOOD PRESSURE
140
120
100
80
60
SUCCESSIVE BEATS

Fig. 1

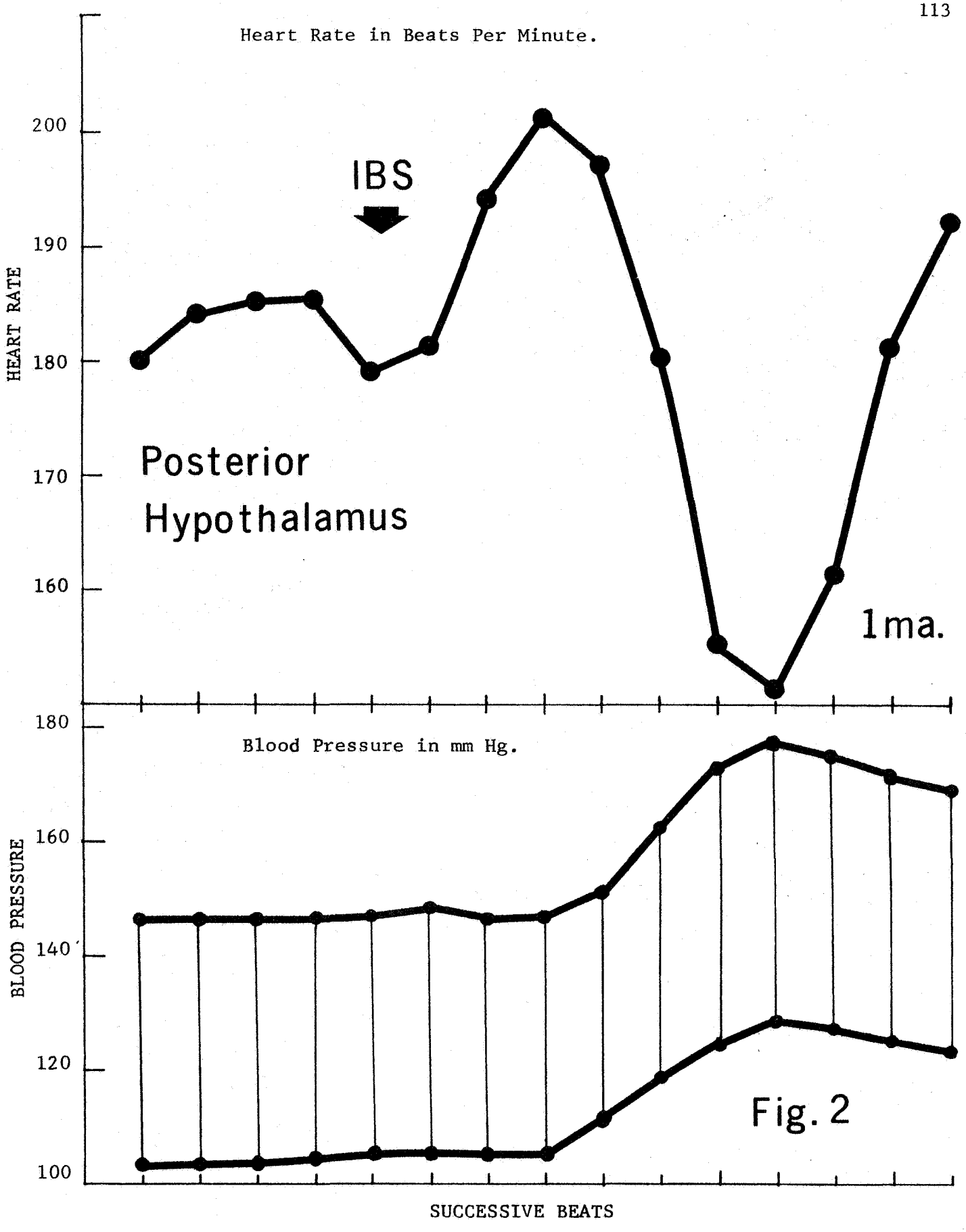
Heart Rate in Beats Per Minute.
HEART RATE
200
190
180
170
160
IBS
Posterior
Hypothalamus
1ma.
180
Blood Pressure in mm Hg.
BLOOD PRESSURE
160
140
120
100
SUCCESSIVE BEATS

Fig. 2

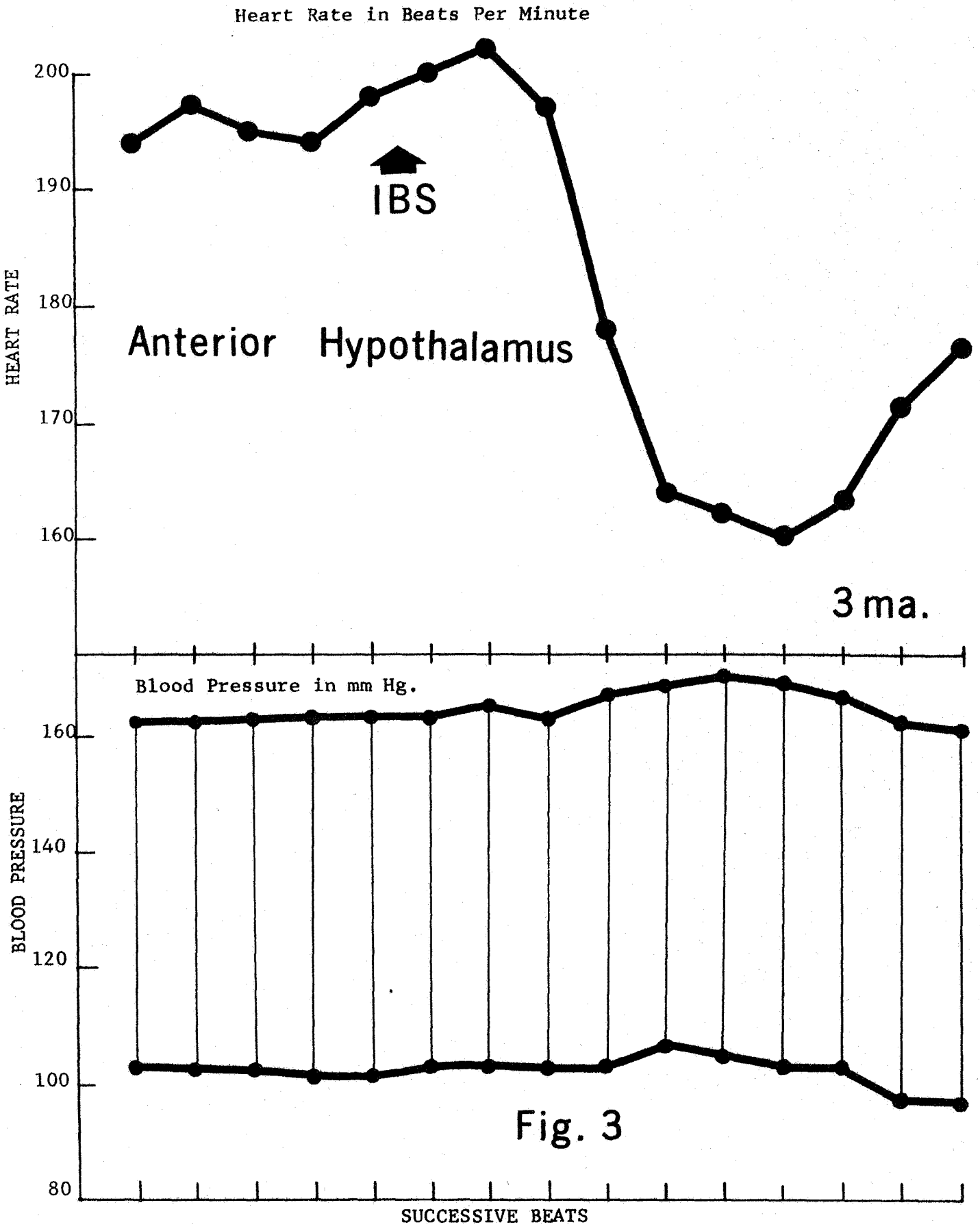
Heart Rate in Beats Per Minute
200
190
180
170
160
HEART RATE
IBS
Anterior Hypothalamus
3 ma.
Blood Pressure in mm Hg.
160
140
120
100
80
BLOOD PRESSURE
SUCCESSIVE BEATS

Fig. 3

AN ATTEMPT TO CONDITION EXTRASYSTOLES USING DIRECT MYOCARDIAL ELECTRICAL STIMULATION AS AN UNCONDITIONAL STIMULUS.

The purpose of this study was to investigate whether extrasystoles, induced "peripherally" by stimulating the ventricular myocardium, can be conditioned. A "peripheral" extrasystoles is one that is not mediated through the nervous system. For extrasystoles which are produced through some emotional, psychic or neurogenic process, we employ the term "centrally mediated". As far as is presently known, extrasystoles which are induced by direct electrical stimulation of the myocardium are not mediated by nervous processes but rather by local excitation of the myocardium at the site of stimulation.

Some investigations have shown that "centrally mediated" extrasystoles can be conditioned (Parmer, 1953; Balanov, 1959; Perez-Cruet, 1962, 1964), and others have emphasized that psychic or emotional factors can induce extrasystoles (Anderson et al., 1939; Katz et al., 1947; Stevenson et al., 1949). The results of these investigations have revealed two primary mechanisms by which centrally mediated extrasystoles are produced. The first is through some primary neural structure such as the hypothalamus or the limbic system via the autonomic nervous system (Beattie et al., 1930; Smirnova, 1961; Porter et al., 1962). The second is by a driving or triggering mechanism in which the central excitation increases the background activity of spontaneous extrasystoles (Royer and Gantt, 1965; Perez-Cruet and Rioch, 1966).

There is no definite proof in the literature of the conditioning of "peripherally" induced cardiac irregularities. Bykov (1957) has shown that extrasystoles occurred when epinephrine was substituted for acetylcholine in dogs which had a conditional reflex electocardiogram to the injection of acetylcholine. He postulated on the basis of this observation that "in actual life, there is every possibility of such collisions when a stimulus combined with the formation of acetylcholine may coincide with the presence of adrenaline in the blood." In the intact animal, experiments with drugs which act peripherally on the heart are difficult to control because most drugs usually have other effects on various organs; they sometimes produce severe hemodynamic changes, and in some instances, can induce behavioral side-effects. Electrical stimulation of the myocardium is a more direct approach to study if peripheral stimulation, without neuronal mediation, is conditionable.

Methods and Materials

Five healthy male dogs, weighing 20 to 30 lbs., were used. They were trained to stand quietly inside a soundproof room - this usually took two to five weeks.

Surgery. After this training, a left or right thoracotomy, depending on the placement of the electrodes, was performed. In three dogs, two electrodes were sutured to the left ventricle, about 1 cm. apart; in another two dogs, in the right ventricle. Figure 1 illustrates the approximate location of the implanted myocardial electrodes.

The electrodes, protected by covering them with an ace bandage and tape, were passed under the skin to the region of the neck. After recovery from the surgery control electrocardiograms were taken. The effects of auditory signals, in the soundproof room, on the electrocardiogram (ECG) and on cardiac

rates were determined before conditioning.

Conditioning Procedure. Classical Pavlovian conditioning procedures were used, and the experiments were run about the same time each day. The animals were presented first with the excitatory CS tone (256 cps in four dogs, 800 cps in one dog) of 5-6 sec. duration, which was reinforced with electrical myocardial stimulation, viz., the unconditional stimulus (US). After an interval of 1-2 minutes the differential (inhibitory) conditional stimulus (CS) was presented. This consisted of a higher frequency tone (512 cps in four dogs, 1,600 cps in one dog, also of 5-6 sec. duration). These tones were not reinforced by the myocardial stimulation.

Stimulation Parameters of the Unconditional Stimulus. The stimulation was bipolar through myocardial electrodes implanted in either left or right ventricle. The electrodes, consisting of strands of stainless-steel wire, were insulated with teflon, down to within a few mm. of the tips. The electrical stimulus to the myocardium consisted of 0.030" train of biphasic rectangular pulses, frequency of 50 cps. The stimulus was triggered by an R wave. The total duration of the unconditional stimulus in which the R waves could trigger stimulation, was approximately 2 to 4 seconds. Pulse duration and delay between positive and negative pulses were of the order of 100 to 500 μsec. The voltage varied from 3 to 4 volts. The approximate electrode resistance varied from 300 to 5,000 ohms.

Apparatus. The programming equipment consisted of transistorized digital logic in which binary counters were used as timing devices for presenting the conditional stimuli. In one system, the actual heart beat was used as a clock with a triggering unit described elsewhere (Perez-Cruet, et al., 1963). In the other system, a precision clock was used for timing instead of the heart beat.

The stimulation of the myocardium of either right or left ventricle was triggered by the R wave of the ECG which activated a triggering unit. The pulse from the triggering unit was shaped for the logical circuit shown in Figure 2. Two one-shot transistorized logic circuits were used (BRS Electronics, Beltsville, Md.). The first one-shot circuit (number 1, shown in Figure 2) was timed with a delay of 150 msecs. or less, at which time a second one-shot circuit (number 2, shown in Figure 2) was activated to keep a relay closed for 20 msec. This relay in turn activated the Tektronix stimulator. (The stimulator consisted of Tektronix components: two, type 161, pulse generators; two, type 162, waveform generators; a monitoring oscilloscope, type 360, and a Tektronix power supply. A transistorized isolation unit was employed to prevent accidental electrical stimulation to the heart.) The electrical stimulation of the myocardium was given after the T waves by adjusting the time delay in the first one-shot circuit (see Figure 2).

An eight channel Offner Type R transistorized polygraph was used to record the various parameters. The electrocardiograms were recorded from three limb leads in the standing position using surface electrodes taped to the right and left foreleg and left hind leg. Limb leads 1, 2, and 3 were recorded from these electrodes. Respiration was recorded using a strain gage respirometer which transduced movement of the thorax into an electrical output which represented respiratory rate. Beat-to-beat heart rate was measured with a Gilford cardiotachometer. The pulse wave was measured with an optical photocell device which measured the luminosity of the animal's skin during each heart beat. The photocell was attached to the skin on the hind leg or the tail. Physiological variables were measured continuously during each experimental session.

Results

None of the dogs showed conditioning of "peripheral" extrasystoles induced by direct electrical stimulation of the myocardium. Three dogs received 100 or more reinforced trials in 5 to 7 different experimental days and another two dogs received 400 or more reinforced trials in 12 to 13 different experimental days, without any evidence of cardiac conditioning.

The dog "Russ" was given in November 1963 during 16 experimental days a total of 1,244 positive conditional stimuli reinforced by unconditional stimulus to the left ventricle. The dog "Lacy" was given for 12 experimental days in January 1964 800 reinforced trials, also to the left ventricle. These were preceded by 120 controls on 3 experimental days. "Jeff" was given 214 reinforced trials (right ventricle) on six experimental days in December 1963 and January 1964, preceded by 40 controls on 26 December. "Brownie" had 50 controls on 21 January 1964 and 438 reinforced trials (right ventricle) in January 1964. "Limon" had 110 controls on 16 and 18 November 1963 and 544 reinforced (left ventricle) trials on 10 experimental days in November 1963.

The results showed that the location of stimulation, whether the right or left ventricle, did not have any influence on the conditionability of "peripheral" extrasystoles. Figure 3 and 4 show no evidence of cardiac conditioning after 104 reinforced trials in Brownie, and 6 reinforced trials in Jeff. The unconditional stimulus (US) was delivered to the right ventricle. Unconditional stimulation of the right ventricle produced extrasystoles with upright R waves in limb leads 1 and 2 and downward-deflection in lead 3 as shown also in Figure 3 and 4. Figures 5,6 and 7 show no evidence of cardiac conditioning after 594 reinforced trials in Russ, 352 reinforced trials in Lacy, and 36 reinforced trials in Limon where the US was delivered to the left ventricle. Unconditional stimulation of the left ventricle produced extrasystoles with downward-deflection of the R waves in limb leads 1, 2 and 3 as shown in Figures 5, 6 and 7.

An interesting finding was that unconditional extrasystoles elicited by stimulation of the left ventricle usually produced vasomotor dilatation, represented by an increase in the amplitude of the pulse volume, after cessation of the unconditional stimulation as shown in Figures 5 and 7, recordings g and e, respectively. In many instances a slowing of the heart rate was observed after left ventricular stimulation. Similar results were observed during unconditional stimulation of the right ventricle in Brownie (see Figure 3).

Four dogs showed muscular twitches of the thorax (costal retraction), during unconditional stimulation of the myocardium but there was never any evidence of pain or discomfort. Thoracic muscular twitches were observed at levels of myocardial stimulation between 2.5 to 5 volts. In two dogs, restlessness was evident prior to myocardial stimulation after 20 reinforced trials (Brownie) and 277 reinforced trials (Lacy) but it subsided quickly. Two other dogs (Limon and Jeff) showed occasional inhibition in the form of sleep even during myocardial stimulation.

Myocardial stimulation in three dogs produced short runs of extrasystoles with ventricular rates ranging from 130 to 210 bpm (see Figures 4, 5 and 7). In two other dogs, ventricular stimulation produced premature ventricular beats more frequently than runs of extrasystoles.

The results showed no clear-cut evidence of heart-rate conditioning, but four dogs showed increments of 9 to 11 heart beats from pre-stimulus levels as shown in Table 1. These heart-rate changes were not differentiated; they may have been associated with a cardiac orienting reflex. Furthermore, one of the dogs (Lacy) showed changes in heart rate during the excitatory conditional stimulus which were indistinguishable from the heart-rate changes during control orienting sessions. Another dog, Jeff, showed an increment in heart rate of 4.3 bpm during the conditional stimuli which was different from the heart rate during the orienting reflex in control sessions.

As we have stated previously, we do not believe that the effect of any agent which is produced by peripheral action solely, without involvement of the central nervous system, can be conditioned (Perez-Cruet and Gantt, 1964). Using atropine and acetylcholine (Thayer and Gantt, 1950; Teitelbaum et al., 1956) to produce tachycardia, histamine for gastric secretions (Katzenelbogen et al., 1939), pilocarpine for salivary (Finch, 1938a) or for prostatic secretions (Finch, 1938b), and adrenaline for hyperglycemia (Gantt et al., 1937), we have been unable to obtain conditional reflexes. When an agent produces some effects throughout the central nervous system and others via the periphery, the central-nervous-system effects can be conditioned whereas the peripheral effects cannot. This partial conditioning is an example of "fractional conditioning" (Fleck and Gantt, 1949).

Our results with peripheral electrical stimulation of the heart are an important extension of previous conditioning studies with drugs which act peripherally and they generalize the application of these studies to other forms of stimulation.

From our work it appears that the criterion for electrocardiographic conditioning is the involvement of the central nervous system in evoking the particular reaction, namely, the mechanisms by which the response is produced rather than the nature of the response itself. Extrasystoles which are mediated by the central nervous system can be conditioned whereas those extrasystoles which are not mediated by the CNS cannot be conditioned.

The conditioning of cardiac irregularities is not possible if there is no possible "neuronal closure" (in the sense in which the Russians use coupling) between the unconditional and the preceding conditional stimuli. These "neuronal closures" can be very critical. For example, not all cardiac irregularities induced by direct stimulation of the hypothalamus can be conditioned (Perez-Cruet, 1963). The "neuronal closure" between the conditional stimulus and the "peripheral" induction of extrasystoles in our stimulation of the myocardium was not accomplished because there is no reflex mediation for "peripherally" induced extrasystoles. The failure to form a myocardial conditional reflex can be explained by the application of a stimulus directly to the myocardium. Thus the effect on the heart is a direct one and not mediated through the central nervous system. Even though there be a representation through feedback, this is not sufficient for the formation of a conditional reflex. The conditional reflex formation requires that the unconditional excitation and the conditional excitation have a common meeting ground in the central nervous system. It is questionable, moreover, whether there was any feedback through afferent impulses from our stimulation of the myocardium.

Clinical impressions in humans with implanted cardiac pacemakers indicate that electrical stimulation of the myocardium is insensible unless extremely high current intensities are used. The fact that no observable heart acceleration was observed after myocardial stimulation in our dogs indicates that the stimulation was not painful. Further support for this impression is that the animals

did not show any evidence of discomfort during stimulation. The costal retraction which was observed in some animals is probably attributed to some irradiation of the current to the costal nerves activating a temporary costal retraction during stimulation. Only two dogs showed restlessness which could be attributed to the costal retraction but this restlessness subsided rapidly.

Previously, we found that conditional tachycardia as a component of food or pain reflexes can be established readily after a few trials (Gantt, 1960), or even after one painful trial (Newton et al., 1961). In contradistinction, the tachycardia produced by myocardial stimulation could not be conditioned in spite of the fact that the number of trials exceeded those normally required for cardiac conditioning of food or pain, and even though the ventricular rates exceeded the heart rate which is usually produced by food or painful stimulation. The explanation of why the tachycardia induced by "peripheral" electrical myocardial stimulation cannot be conditioned whereas the tachycardia to food or painful stimulation can, rests upon that fact that the last is "centrally mediated."

The phenomenon of vasodilatation observed after myocardial stimulation can be explained on the basis of the cardiac slowing, however, the mechanism for the cardiac slowing after stimulation is yet to be studied. Recent studies in surgically blocked dogs, where the heart rate was maintained with pacemakers, have shown a transient ventricular arrest upon cessation of the ventricular pacing (Brockman, 1965). The mechanism for this effect is probably hemodynamic because if these changes were of a reflex nature, some form of conditioning would have been observed in our dogs.

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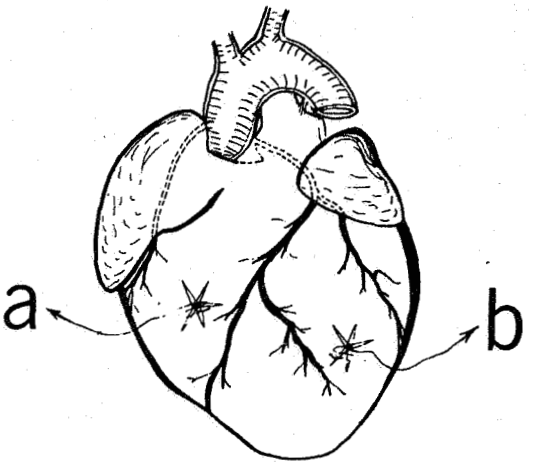

Fig. 1. Location of myocardial electrodes in the (a) right ventricle and (b) left ventricle.

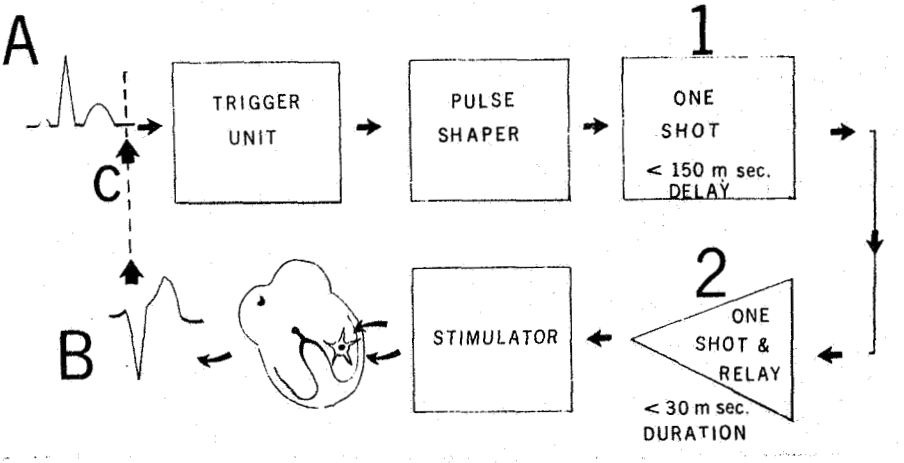

Fig. 2. Block diagram showing the design of the synchronizing circuit for electrical stimulation after the depolarization of the ventricles (t waves). Arrows indicate the direction of electrical and physiological events.

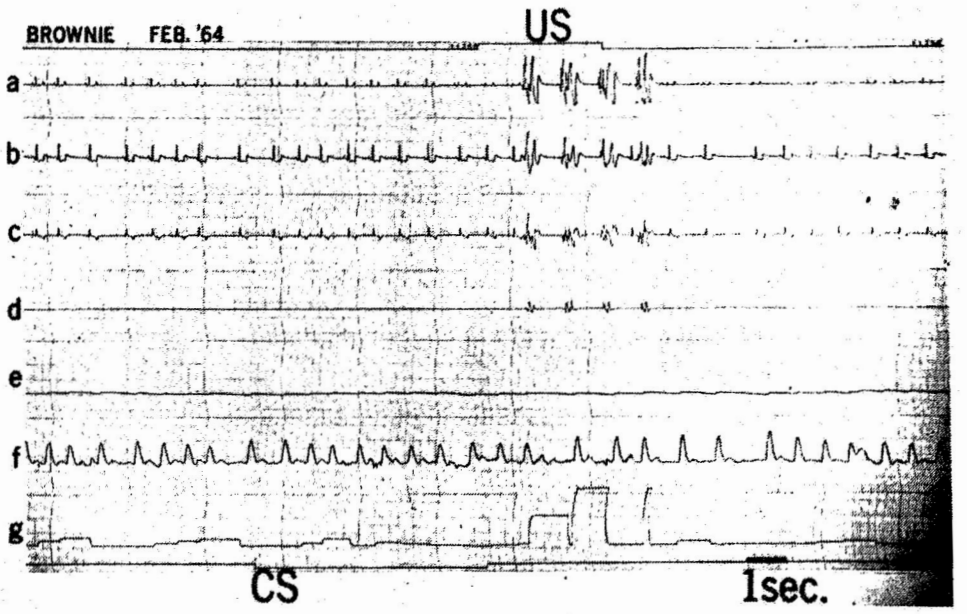

Fig. 3. Polygraph tracing in Brownie showing no conditioning of extrasystoles after 104 reinforced trials. CS stands for a 6 sec. conditional stimulus (T 256). US stands for unconditional stimulation which in this case was given in the right ventricle (see run of extrasystoles). a,b, and c, electrocardiograms from limb leads 1, 2 and 3. d, unconditional stimulus marker, e and f, show DC (background luminosity) and AC (pulsatile) optical pulse volume waves. g, beat-to-beat heart rate.

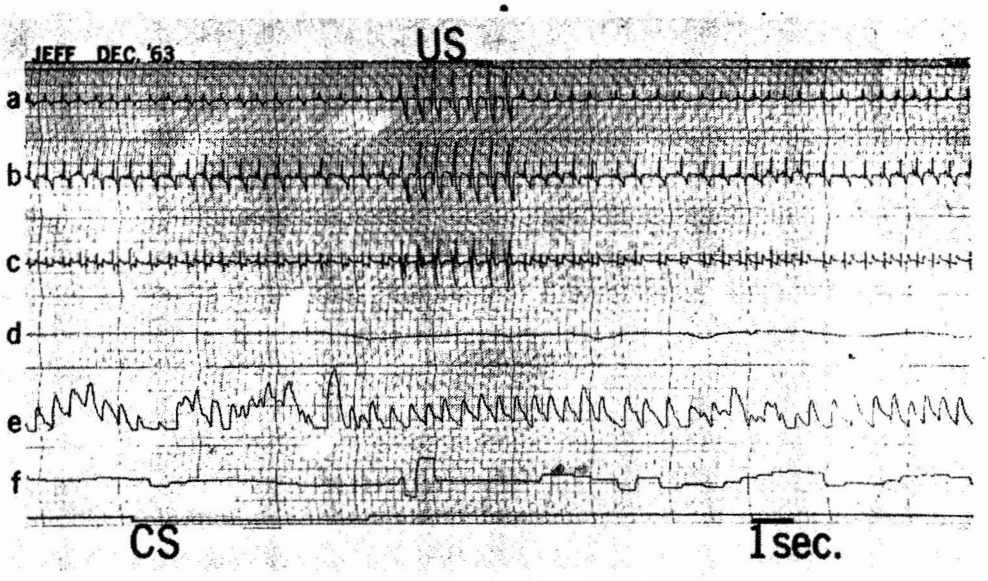

Fig. 4. Tracing in Jeff showing no conditional extrasystoles in trial No. 6. a, b and c, electrocardiograms from leads, 1, 2 and 3. d, respiration. Note a slight increment in respiratory rate after US. e, pulse volume waves. f, beat-to-beat heart rate. CS, conditional stimulus (T 256). US, unconditional stimulus to the right ventricle.

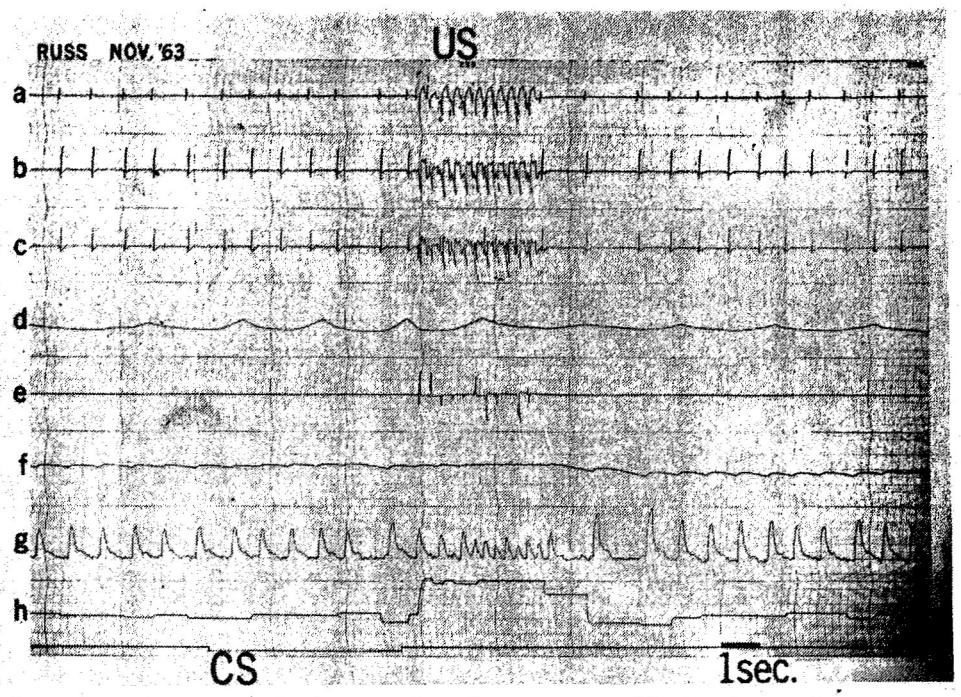

Fig. 5. Tracing in Russ showing no conditioning of extrasystoles after 594 reinforced trials, a, b and c, electrocardiograms from limb leads 1, 2 and 3. d, respiration. Note a slowing of respiration after US. e, unconditional stimulus marker. f and g, DC and AC optical pulse volume waves. Note a slowing of the heart rate and an increase in the pulse volume amplitude after US. h, beat-to-beat heart rate. CS, a 5 sec. conditional stimulus (T 800). US, unconditional stimulus to the left ventricle.

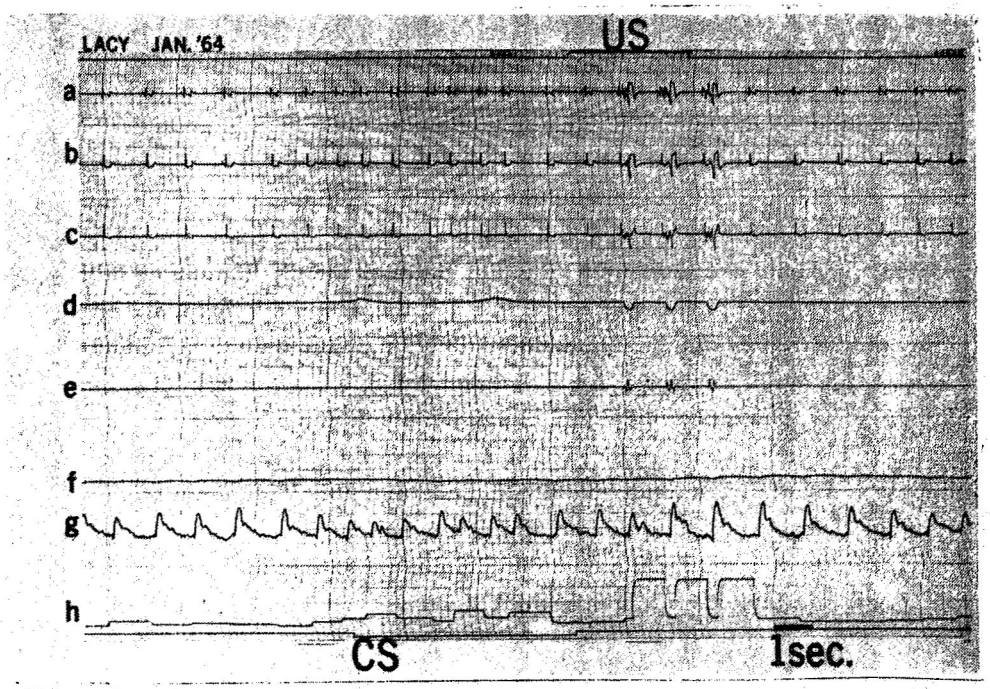

Fig. 6. Tracing in Lacy showing no conditioning of extrasystoles after 352 reinforced trials, a, b and c, electrocardiograms from limb leads 1, 2 and 3. d, respiration. e, unconditional stimulus marker. f and g, DC and AC optical pulse volume waves, h, beat-to-beat heart rate. CS, conditional stimulus (T 256). US, unconditional stimulus to the left ventricle.

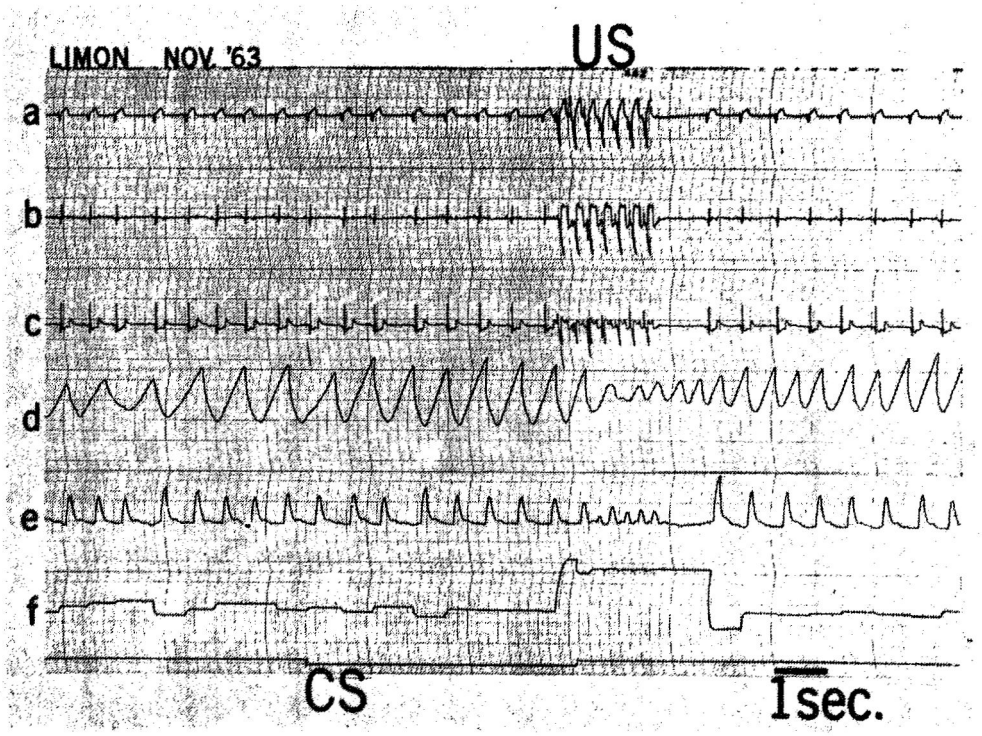

Fig. 7. Tracing in Limon showing no conditioning of extrasystoles after 36 reinforced trials. a, b and c, limb leads 1, 2 and 3. d, respiration Note an increase in respiratory rate even though there is cardiac slowing. e, AC optical pulse volume waves. f, beat-to-beat heart rate. CS, conditional stimulus (T 256). US, unconditional stimulus to the left ventricle.

TABLE 1. Average Heart-Rate Changes during Attempted Conditioning of Extrasystoles Using Direct Myocardial Stimulation as an Unconditional Stimulus

Dog	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
	N	Before	During	After	N	Before	During	After
Russ								
CR Data	622	64.5	76.5	70.4	604	64.1	75.7	70.3
Lacy								
OR Data	60	90.0	99.6	93.6	60	87.8	96.8	90.1
CR Data	400	77.3	87.1	86.7	400	76.3	87.2	78.4
Limon								
OR Data	51	68.8	73.9	71.1	51	68.8	72.5	70.1
CR Data	272	68.0	78.7	75.6	272	72.8	78.4	74.6
Brownie								
OR Data	25	99.2	103.6	98.4	25	96.8	93.6	97.2
CR Data	225	81.1	90.0	88.0	225	80.5	88.1	85.6
Jeff								
OR Data	25	109.2	105.2	108.8	25	103.6	103.2	100.0
CR Data	103	115.7	120.0	122.7	103	115.0	119.6	118.5

Explanation of Table 1

Columns 1 and 5 show the number of trials counted.
Column 2 is the HR during 5-6 sec. before the onset of the orienting or conditional signal.
Column 3 is the HR during the CS.
Column 4 is the HR after the OR or after the CR.
Column 6 is the HR for the 5-6 sec. before the OR or before the Inhibitory tone.
Column 7 is the HR during the OR or during the Inhibitory CS.
Column 8 is the HR 5-6 sec. after the OR or after the Inhibitory tone.

HR is Heart Rate in beats per minute.

CARDIOVASCULAR CONDITIONING USING HYPOTHALAMIC STIMULATION AS AN UNCONDITIONAL STIMULUS.

Changes in cardiac rate and rhythm, including extrasystoles, induced by stimulation of vagus or sympathetic nerves have been amply demonstrated by Rothberger and Winterberg (1910) and Andrus and Carter (1930). Stimulation of the hypothalamus elicits tachycardia or premature beats according to Beattie, Brow and Long (1930); Weinberg and Fuster (1960); and Hess (1957). Cardiac irregularities, including extrasystoles, have been elicited by conditional reflexes in man by Perez-Cruet (1962). Conditional changes in the electrocardiogram including amplitude of T wave independent of the accompanying tachycardia have been reported after repeated injections of bulbocapnine, a centrally acting drug by Perez-Cruet and Gantt (1964).

The main purpose of the present study is to determine whether cardiovascular responses induced by electrical stimulation of the hypothalamus can be conditioned. If a coupling can be established between a conditional stimulus and electrical stimulation of the hypothalamus, basic principles of psychogenic influences on the cardiovascular system could be elucidated with this experimental model.

In 14 dogs intracerebral electrodes were implanted in the hypothalamus and other subcortical areas using a Baltimore stereotaxic instrument and a stereotaxic atlas of the dog's brain. Electrical stimulation was performed using a Tektronic stimulator and a transistorized isolation unit. All experiments were carried out in a soundproof room. The subjects were observed through a one-way mirror and on closed-circuit television. Electrocardiogram, using standard limb leads (I, II, and III), respiration, and beat-by-beat changes in heart rate were recorded simultaneously throughout the experiment. In 6 dogs, direct blood pressures were measured by an intra-arterial catheter. In some dogs, plethysmographic recordings, using an optical pick-up for pulse changes, have been taken and pupillary changes have been measured.

The parameters of stimulation consisted of bidirectional square waves of 100 cy/sec.; trains of biphasic pulses were presented for 0.5 to 1.0 second; current level varied between 0.5 to 3 ma. The effects of the electrical stimulation at different intensities were tested in all intracerebral placements a few days after surgery.

Conditioning was performed using established procedures in which a conditional stimulus (a tone of a given frequency such as 256, 400 or 1600 cycles per second for 6 seconds) was reinforced at the end with electrical stimulation to the hypothalamus.

All dogs displayed unconditional cardiovascular changes upon electrical stimulation of the hypothalamus. Electrocardiographic inrregularities such as extrasystoles, nodal beats, escape, AV block and Wenckebach phenomenon* were observed.

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* Periods in which P-R (A-V interval in the ECG lengthens progressively until a QRS complex (Ventricular response) drops out, whereupon the periodic pattern is repeated.

Figure 1 illustrates the development of nodal ectopic beats and ventricular extrasystoles, viz. the unconditional ectopic beats, in the first 50 reinforcements to the hypothalamus in the dog, Romulus. Note that 4 to 12 seconds after electrical stimulation of the hypothalamic placement nodal beats predominate (150 to 210 or 3 to 4 per 4 seconds intervals). Ventricular extrasystoles appear more frequently (about 1 every 4 seconds) twenty seconds after hypothalamic stimulation as an unconditional stimulus.

Figure 2 illustrates a tracing of Wenckebach phenomenon after hypothalamic stimulation in another dog, Neron.

Increases in the amplitude of the T wave were recorded in five dogs. The heart rate usually accelerated from 40 to 100 beats above control levels. Changes in blood pressure ranged from 40 to 150 mm Hg. above control levels observed prior to stimulation. Respiratory changes varied, but usually the respiratory rate was accelerated. The amplitude of the plethysmogram was usually decreased after stimulation (vasoconstriction).

Cardiovascular conditioning to hypothalamic stimulation has been observed in nine dogs. (A) Clear-cut electrocardiographic conditioning has been observed in two dogs; one dog has shown conditioning of the Wenckebach phenomenon (see Figure 3) which was elicited by hypothalamic stimulation; another has shown conditioning of nodal rhythms and ventricular extrasystoles (see Figure 4). Three dogs have shown transient conditioning of the T wave changes, similar to those observed in the conditional reflex to bulbocapnine (Perez-Cruet and Gantt, 1964). Two other dogs, which developed extrasystoles in about 25-40% of the reinforced trials, did not show conditioning. (B) Heart rate conditioning has been observed in six dogs; in three dogs heart rate conditional reflexes were manifested as a severe supraventricular tachycardia. Figure 5 illustrates conditional HR-CR, BP-CR and respiratory CRs to hypothalamic stimulation during a non-reinforced (-CS) and reinforced (+CS) conditional stimuli. (C) Blood-pressure conditioning has been evident in four dogs showing transient conditional changes in systolic and diastolic blood pressures. In one dog, conditional blood pressure changes were studied for 12 months. There was no evidence that persistent hypertension had been produced by applying hypothalamic stimulation as an unconditional stimulus intermittently over that period. However, transient hypertension in the experimental environment was established and this reaction could not be extinguished after 3,000 unreinforced trials (see Figure 6).

Histological examination of the brain has been performed in four dogs. In one dog, in which conditional electrocardiographic changes were observed, the tip of the electrode, from which cardiovascular changes were elicited, was located in the dorsal part of the dorsomedial nucleus of the hypothalamus. In another, in which heart rate conditioning was observed, the tip of the electrode rested in the posterior hypothalamus immediately adjacent to the anterior mammillary nucleus.

The study reveals that cardiovascular reactions, such as electrocardiographic abnormalities (Perez-Cruet, 1963), blood pressure and heart rate changes, induced by hypothalamic stimulation can be conditioned. The study suggests that electrocardiographic conditioning can be mediated by excitation of supra-medullary nervous structures. In our experiments this excitation was produced directly by electrical stimulation. On the basis of our findings one can speculate that it is possible for active neural foci to get excited by natural neural impulses and that a coincidence between external stimuli and the development of these natural active foci within the hypothalamus or limbic structures can establish or bring out a "coupling" between the external stimuli and neurally mediated

irregularities; thus producing conditional cardiac irregularities and possibly in extreme cases cardiac death. Work is now in progress to determine whether similar cardiovascular changes from other brain areas (structures related to the limbic system) which are known to elicit cardiovascular changes, can also be elaborated as conditional reflexes.

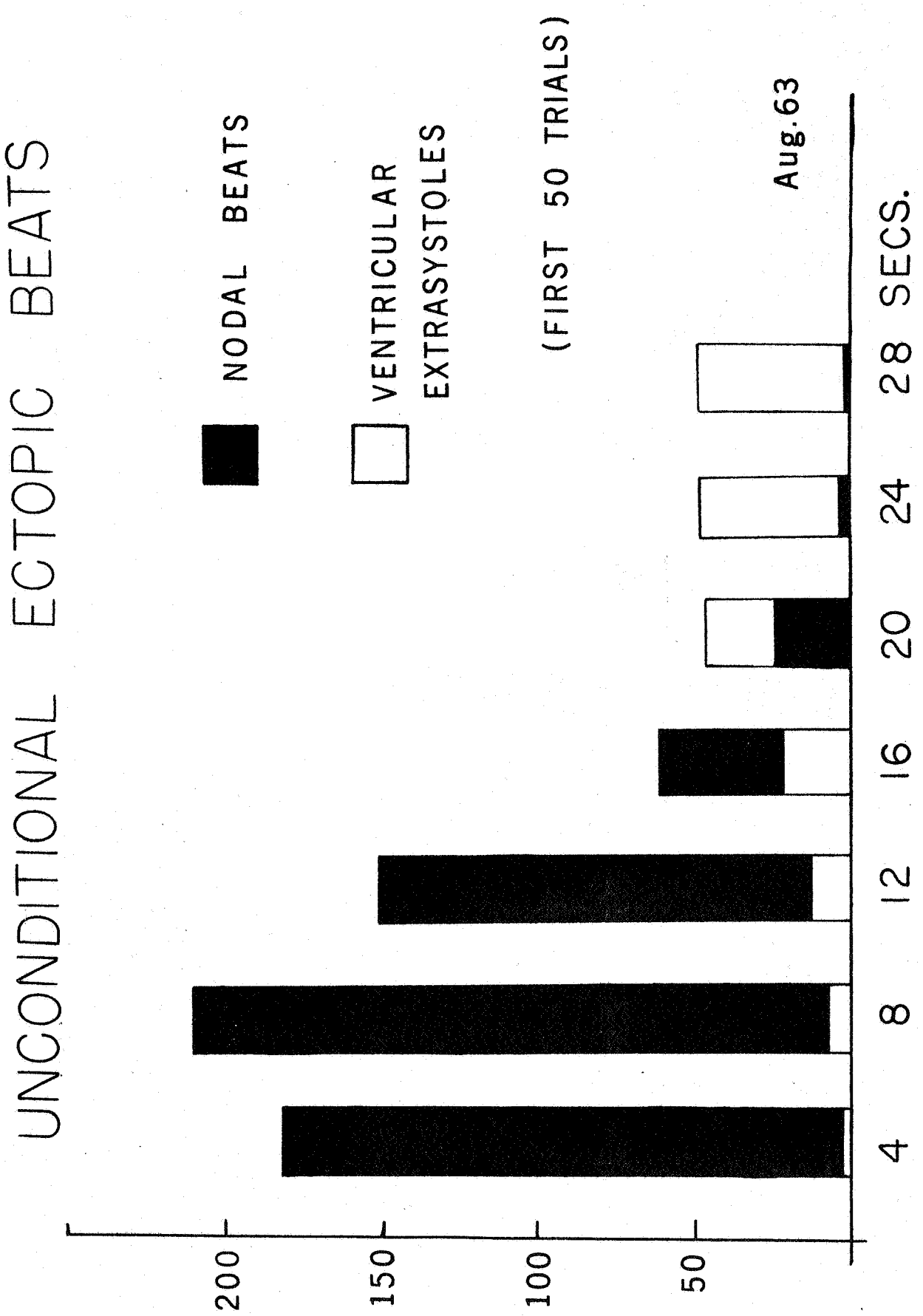

UNCONDITIONAL ECTOPIC BEATS
NODAL BEATS
VENTRICULAR EXTRASYSTOLES
(FIRST 50 TRIALS)
Aug.63
200
150
100
50
4
8
12
16
20
24
28 SECS.

FIGURE 1

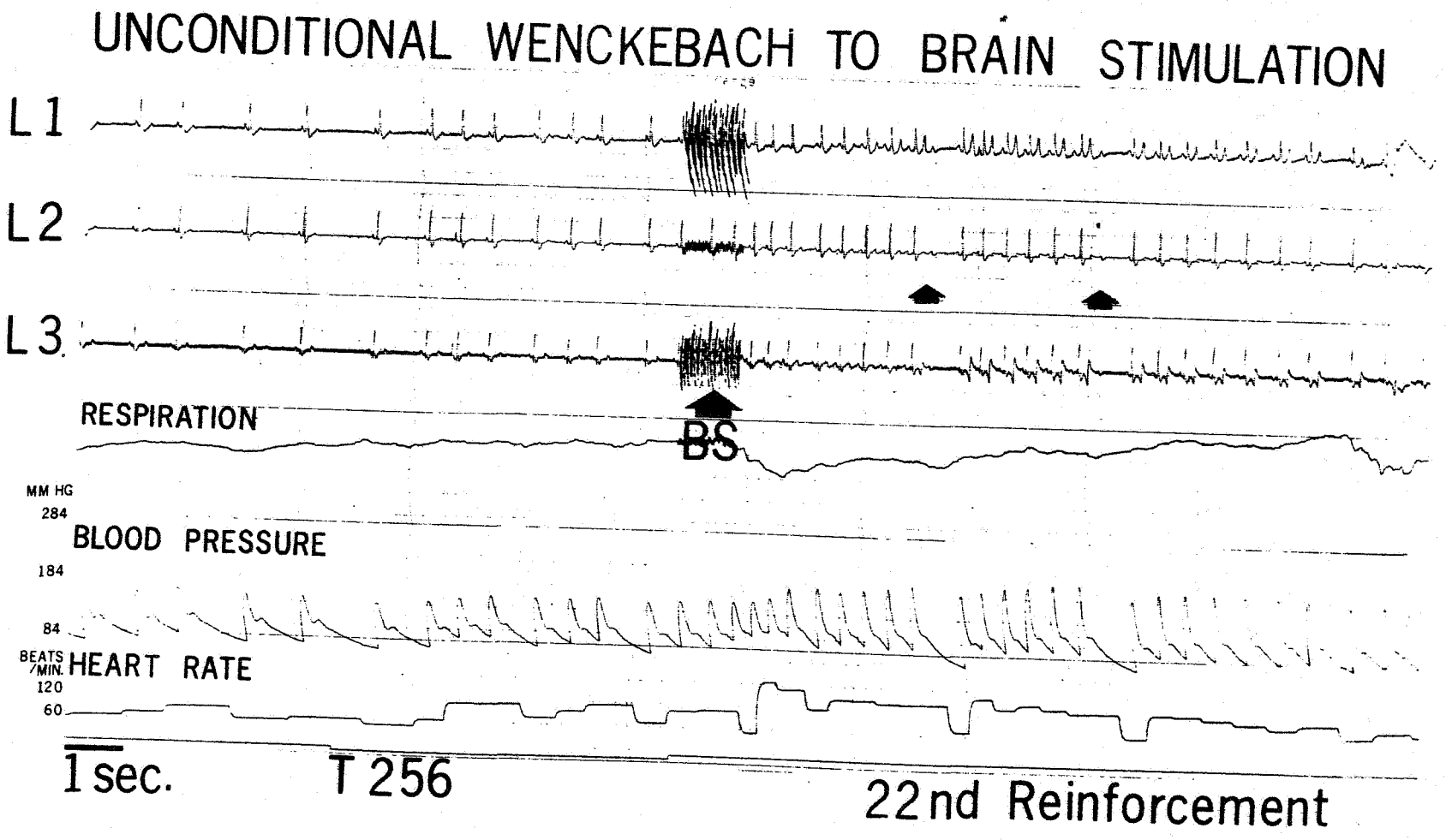

UNCONDITIONAL WENCKEBACH TO BRAIN STIMULATION
L1
L2
L3
RESPIRATION
BS
MM HG
284
BLOOD PRESSURE
184
84
BEATS /MIN.
HEART RATE
120
60
1 sec.
T 256
22nd Reinforcement

FIGURE 2

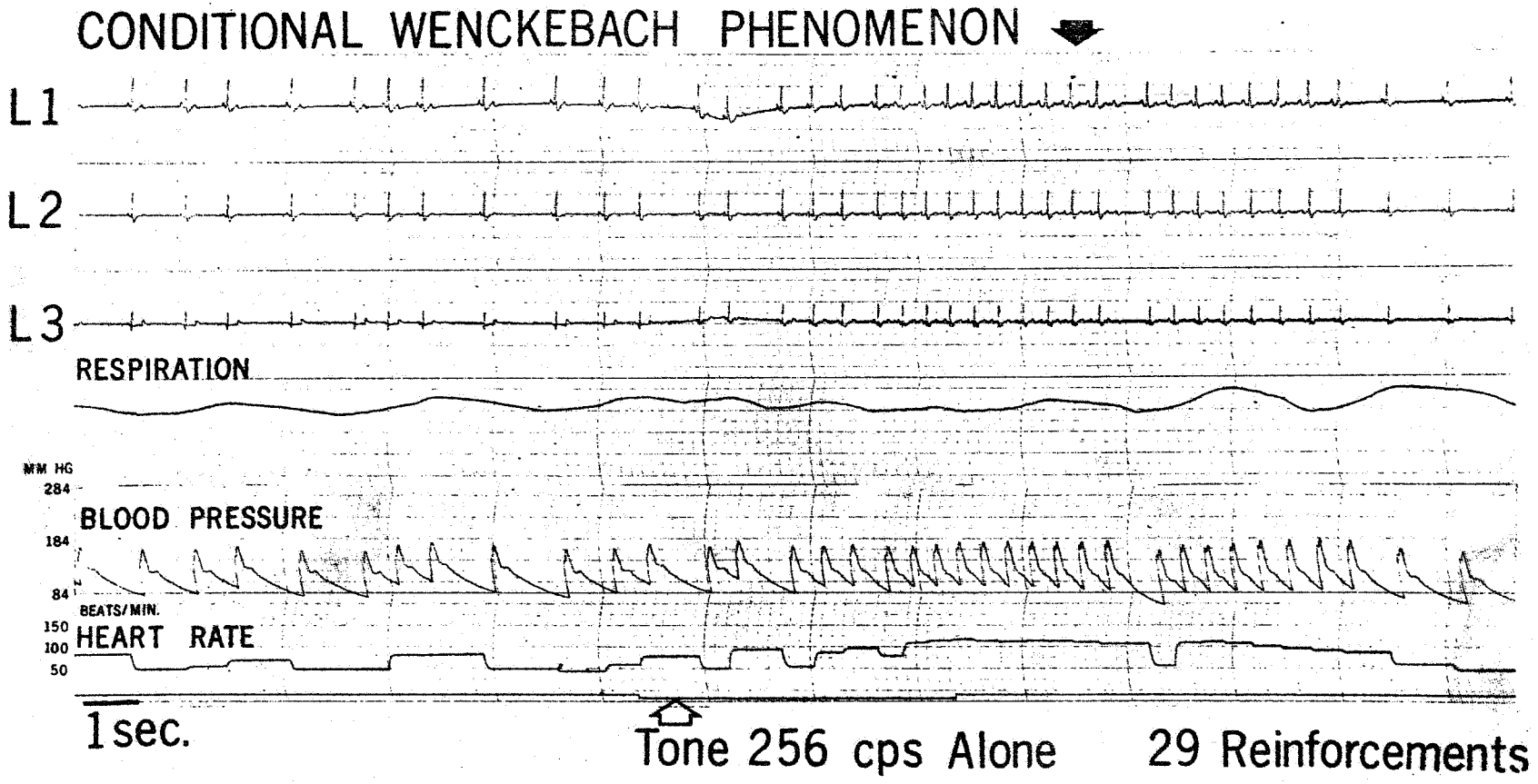

CONDITIONAL WENCKEBACH PHENOMENON
L1
L2
L3
RESPIRATION
MM HG
284
BLOOD PRESSURE
184
84
BEATS/MIN.
150
100
HEART RATE
50
1 sec.
Tone 256 cps Alone
29 Reinforcements

FIGURE 3

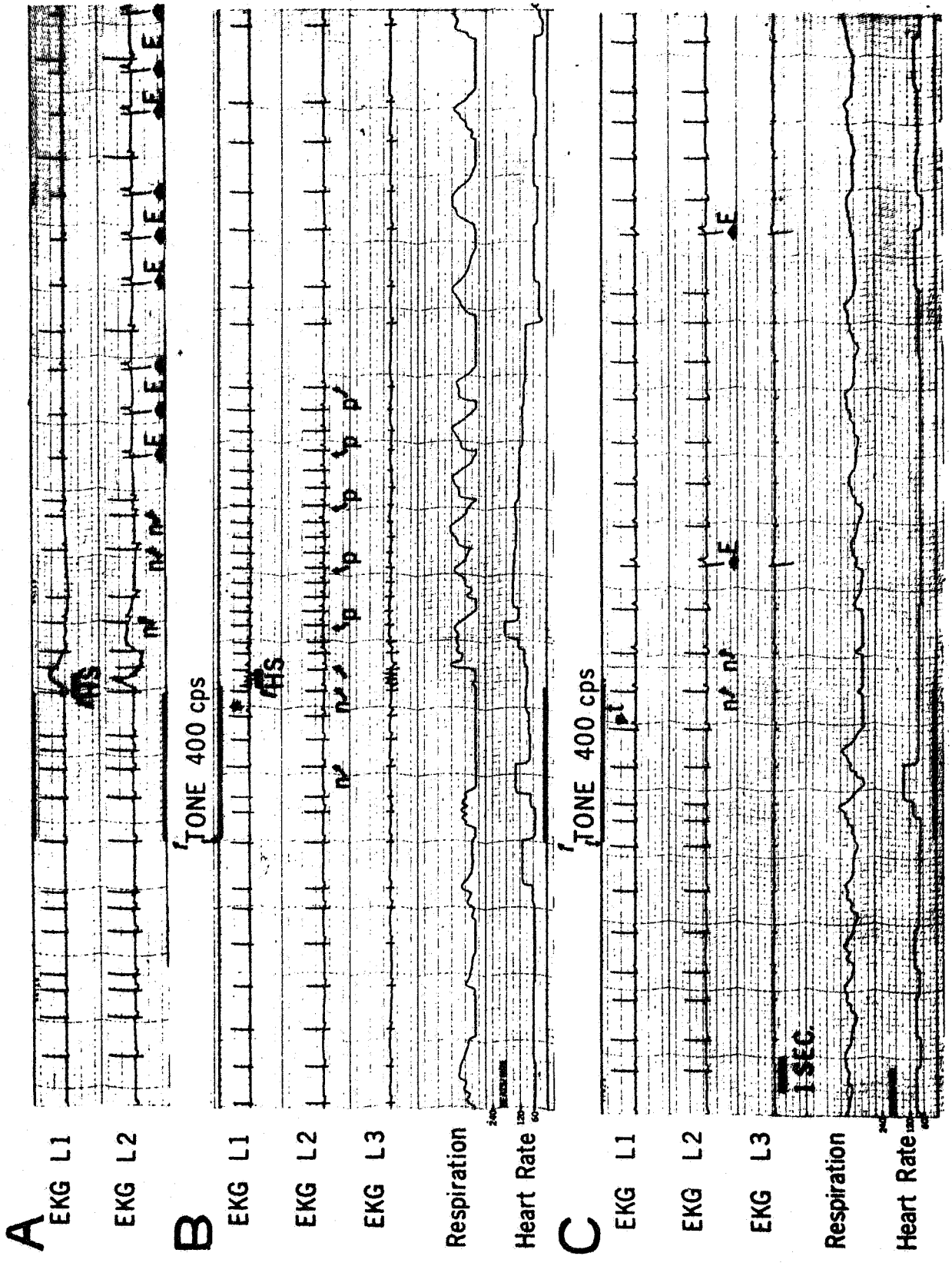
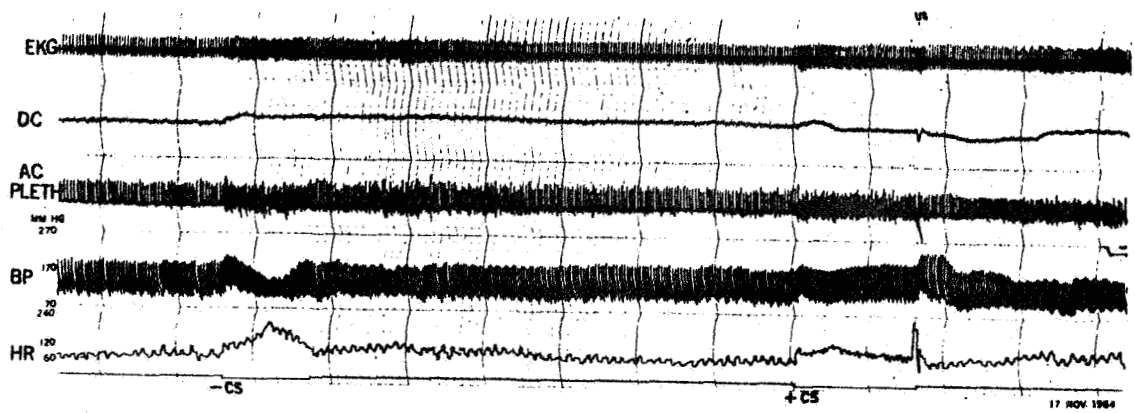

A
EKG L1
EKG L2
HS
B
EKG L1
EKG L2
EKG L3
Respiration
Heart Rate
TONE 400 cps
HS
C
EKG L1
EKG L2
EKG L3
Respiration
Heart Rate
TONE 400 cps
1 SEC.

FIGURE 4

A.



B.

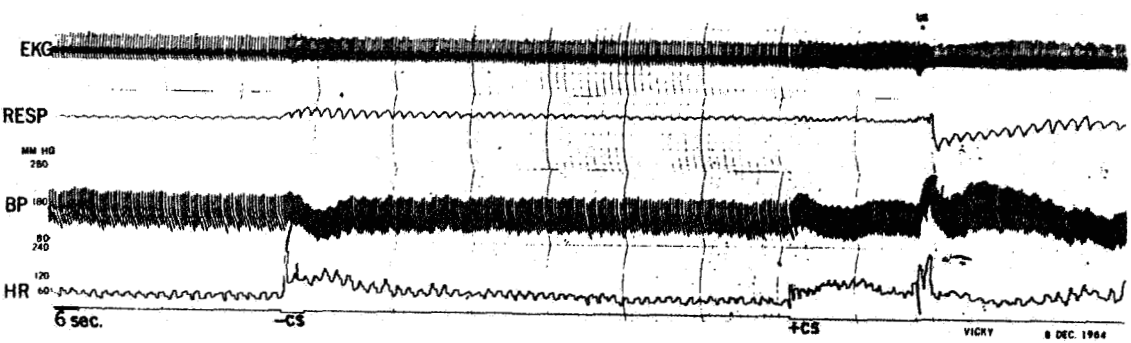


Fig. 5. Polygraph tracing A and B illustrating EKG, DC and AC optical plethysmography, intra-arterial blood pressure, and heart-rate beat-by-beat. During -CS (T512), there is an acceleratory HR-CR (A, control: 80bpm; -HR-CR: 200 bpm). During +CS (T256) which was always reinforced with hypothalamic stimulation there is also a HR-CR (A, control: 80 bpm; +HR-CR: 120 bpm). The tracings also illustrate BP-CR is represented by a decrease about 40 mm Hg below the pre-stimulus BP levels. During the +CS, the BP-CR consist of a slight increase in BP (20 mm Hg above pre-stimulus levels). Hypothalamic stimulation, marked US, produced a sudden but short lasting acceleration in HR and an increase in blood pressure (30 to 40 mm Hg).

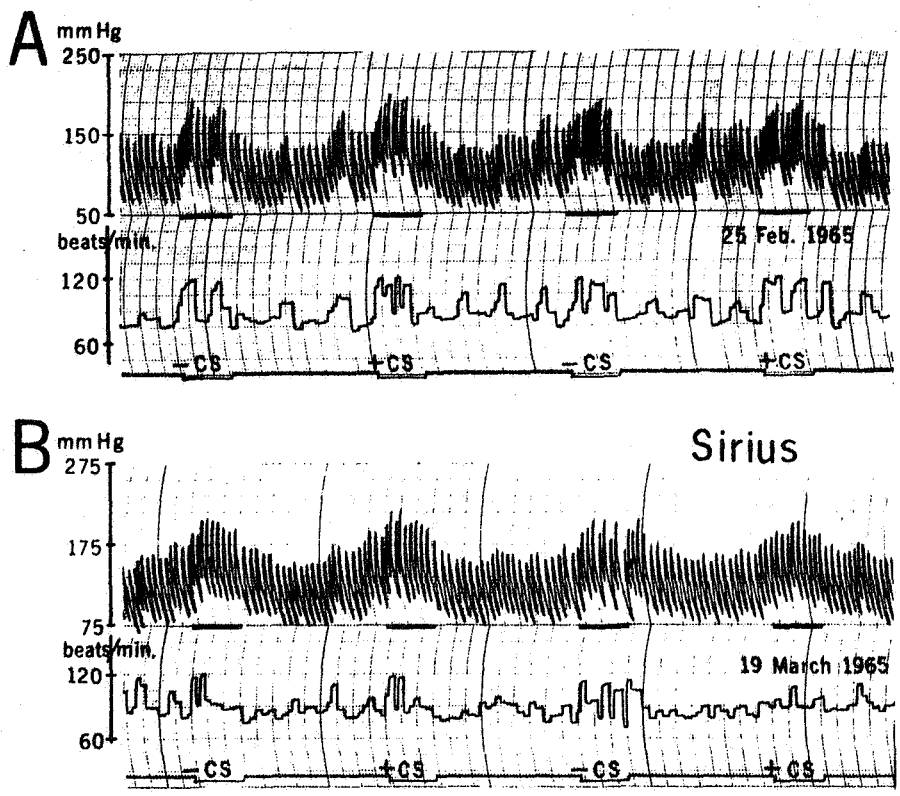


Figure 6: Blood pressure and instantaneous heart rate responses to an auditory conditional stimulus during extinction. A. cardiovascular conditional responses at the beginning of extinction. B. after 3,000 extinction repetitions of the conditional stimuli. +CS excitatory tone (T256 or T800). -CS inhibitory tone (T512 or T1600). Paper speed was 5 mm./sec..

CARDIOVASCULAR FUNCTIONS AS AN INDEX OF PAVLOVIAN INHIBITION.

Pavlovian inhibition is a complex nervous process which is the resultant of numerous psychophysiological activities in an organism. Pavlov (1928) described the process of inhibition as external when it originated from sources outside of the specific field of excitation or as internal when it originated from within the specific field of excitation. Pavlov considered inhibition as a "braking process" which manifests itself as a diminution or disappearance of conditional reflexes (CR). The process of inhibition can be seen in some conditional autonomic functions as shown by Alexajan (1958) and Pickenhain (1959).

Gantt (1947) has tried to use the heart rate (HR) as an index of inhibition during inhibitory CR, viz. inhibitory cardiac CR, because negative phases of inhibition below a baseline could not be detected with secretory CR, such as salivary CR, because the secretion never reaches a negative value.

The purpose of this study is twofold: to determine whether cardiovascular functions can be used as an index of Pavlovian inhibition and to explore the usefulness of the cumulative method of illustrating the development of internal inhibition in the form of inhibitory cardiac CR.

Material and Method

Ten dogs were trained with a classical defensive conditioning method used previously (Gantt, 1944). The dogs were placed in a soundproof room and they were observed through a one-way mirror in order to avoid stimuli other than the conditional stimuli from interfering with the experiments.

Two tones were used as conditional stimuli (CS). A 6-second tone, 256 cy/sec., was used as an excitatory CS always reinforced with electric shock to the left foreleg at the end of the CS. Another 6-second tone, 512 cy/sec., was used as an inhibitory CS which was never reinforced. The intertrial interval between the excitatory and inhibitory tones was 2 minutes. In all dogs, extinction of the orienting reflex (OR) was carried on before conditioning.

Heart rate (HR) was measured with a Gilford cardiotachometer. EKG was recorded concurrently with the analog HR output from the cardiotachometer. Concurrent recording of the EKG was essential for cross-checking and validation of the analog HR output from the cardiotachometer. The R wave from the EKG was used to trigger the Gilford cardiotachometer using a triggering unit described elsewhere (Perez-Cruet, et al., 1963). In some dogs, beat-to-beat HR changes were analyzed with an EKG ruler and averaging was done using a method described by Newton and Perez-Cruet (1965). Respiration was monitored in some dogs with a circumthoracic strain gage belt. Motor CRs were also measured and graded in terms of strength of foreleg flexion.

The analysis of the data in terms of cumulative tracings was done using the analog HR tracing from the Gilford cardiotachometer. The cardiotachometer was calibrated using ranges of 60, 120 and 240 beats per min. HR deceleration and acceleration were represented respectively by a decrease or increase in the linear output from the cardiotachometer. In this study we measured the inhibitory process in terms of HR deceleration. HR acceleration was usually observed with the excitatory CS, Tone 256. For that reason, analysis of the inhibitory process was done only during the non-reinforced inhibitory CS, that is, Tone 512.

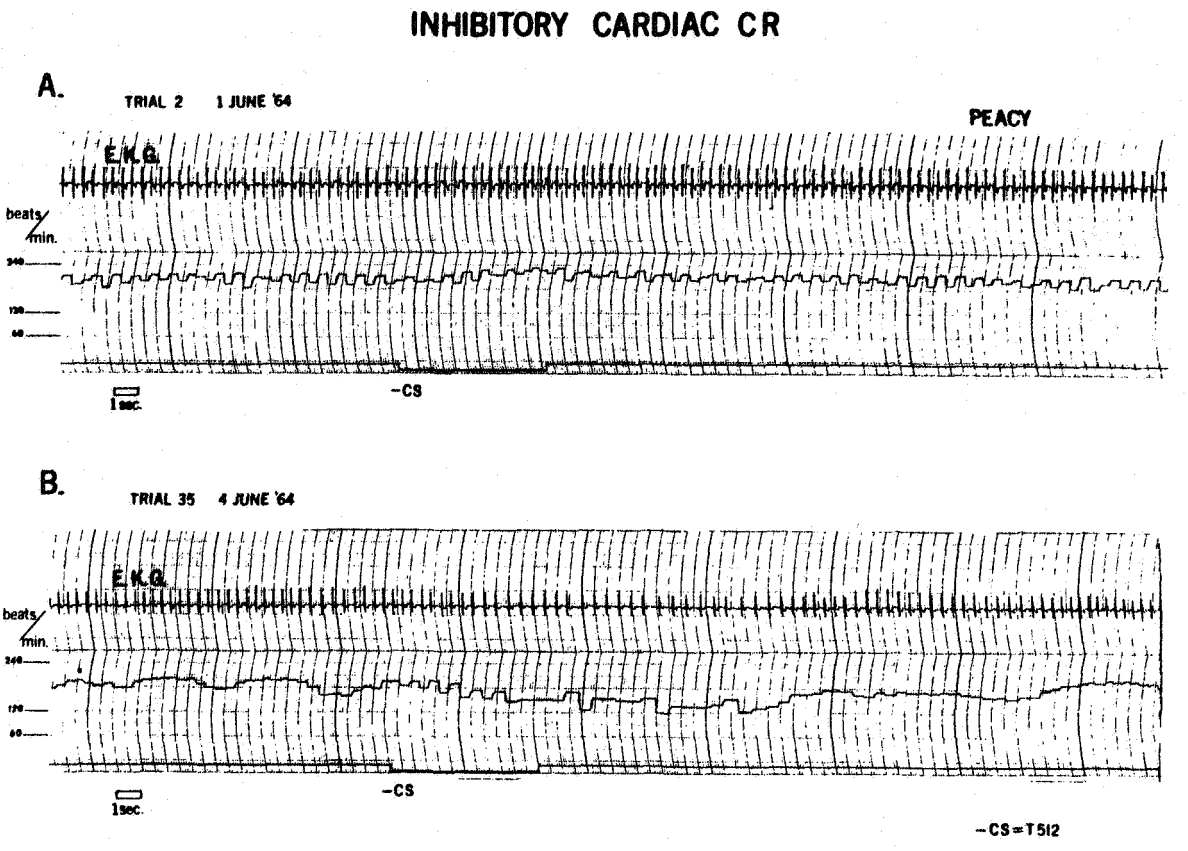


Figure 1: Tracings A & B illustrates EKG and analog HR output from the Gilford cardiotachometer. At A, tracing represents the 2nd non-reinforced trial. Note that during non-reinforced tone (-CS) there is an acceleration in HR represented by an increase in the linear output from the cardiotachometer. At B, tracing represents the 35th non-reinforced trial, Note that during the non-reinforced tone (-CS) there is a deceleration in HR represented by a decrease in the linear output from the cardiotachometer.

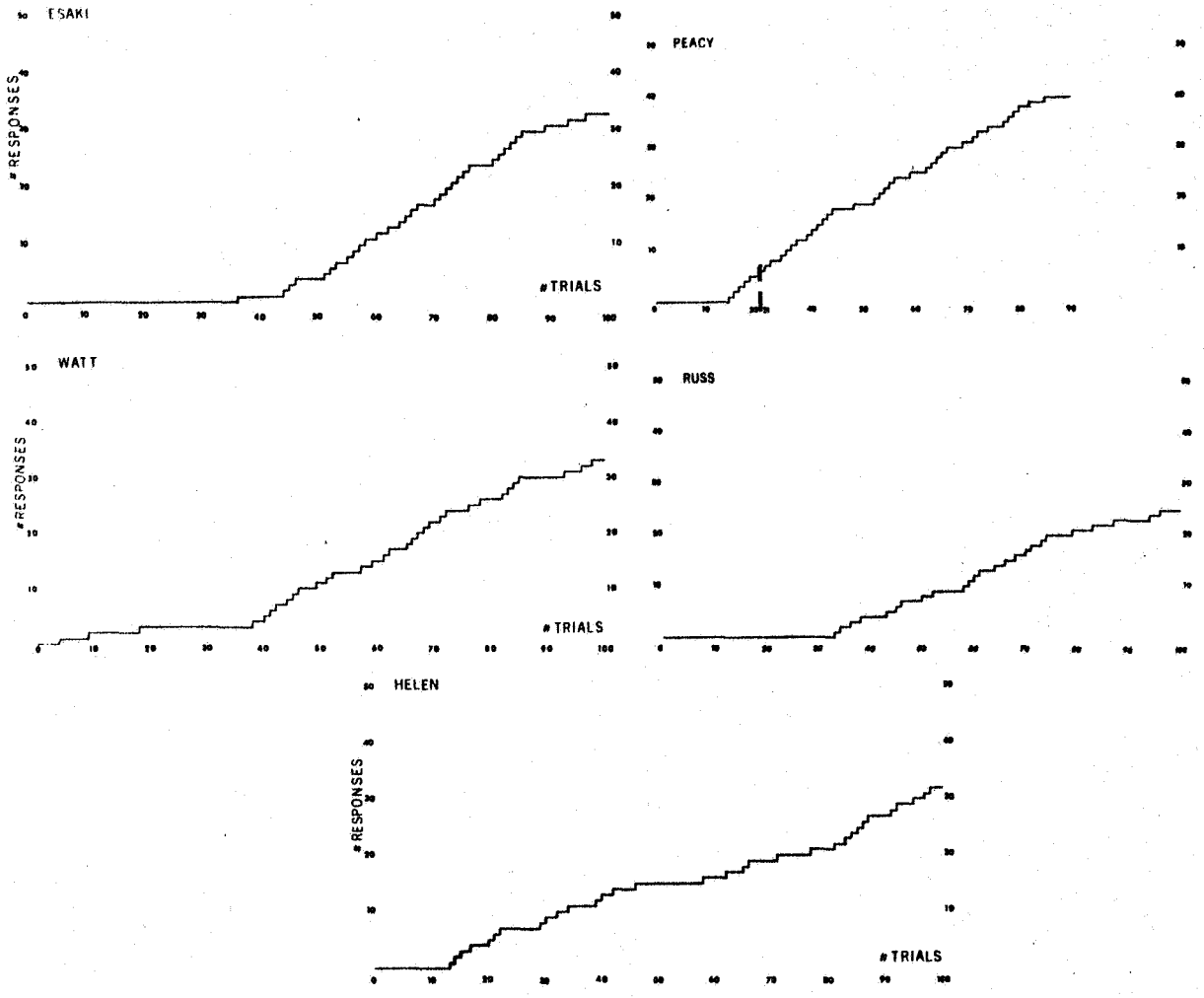


Figure 2: Cumulative tracing showing the establishment and development of active cardiac inhibition in five dogs. Note that inhibitory cardiac CRs appeared between the 14th and 45th non-reinforced trials after beginning of conditioning. Notice that the inhibitory process is active once it is established.

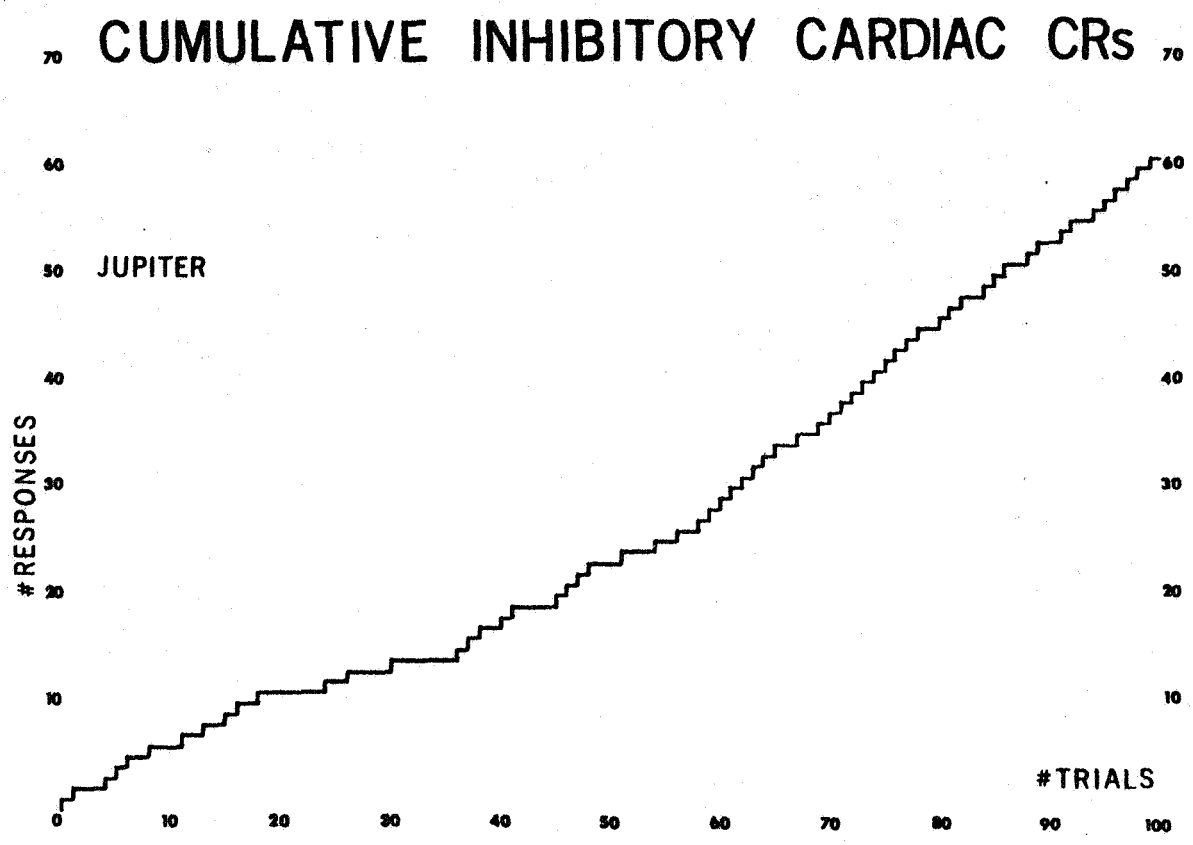


Figure 3: Tracing illustrating inhibitory cardiac CRs from the beginning of conditioning in Jupiter. Note that the inhibitory process leveled between non-reinforced trials 20 and 38 and reappeared actively after the 40th non-reinforced trial.

A deceleration in HR, during or immediately after the inhibitory, CS was recorded as an inhibitory cardiac CR. The inhibitory cardiac CR was plotted on graph paper with ordinate indicating cumulated responses and in the abscissa trial number. Every inhibitory cardiac CR was cumulated one single step per response along the ordinate. Trials in which there was an acceleration or no changes in HR were not cumulated but they were also recorded with the inhibitory trials along the abscissa. The first 90 to 150 trials were examined and plotted.

Results:

The results showed a 70% incidence of HR deceleration during or after the inhibitory tone 512 as conditioning progressed in our series.

Figure 1 illustrates actual tracings of the analog HR output from the Gilford cardiotachometer during inhibitory tone 512 after excitatory tone 256 (not illustrated in the figure) has been reinforced 2 and 35 times respectively. Notice that in tracing A after 2 reinforced trials there is a slight increase in HR during and after the inhibitory CS indicating generalization of the excitatory cardiac CR and lack of differentiation. In tracing B, after 35 reinforced trials there is a deceleration in HR during and after inhibitory CS, tone 512. The deceleration in HR in this tracing was recorded as an inhibitory cardiac CR and plotted as a response in the cumulative tracings.

In 5 dogs the inhibitory process was detected in the HR between non-reinforced trials 14 and 45 after the beginning of conditioning, as shown in Figure 2. The overall percentages of cumulated responses at the end of 100 trials varied between 25% and 40%. The slope of the cumulative tracings was more reliable in illustrating the establishment of the inhibitory cardiac CR than the absolute values in overall percentages (see Figure 2)

Another dog showed inhibitory cardiac CR immediately after the first trial but the process disappeared between non-reinforced trials 20 and 38 and reappeared after the 40th non-reinforced trial. The percentage of cumulated responses at the end of 100 trials was 60% (see Figure 3). Furthermore, this animal could be typed behaviorally as an inhibitory type showing weak motor CRs.

The HR deceleration was observed during extinction of the orienting reflex to tone 256 and 512. Figure 4 illustrates 75 trials of orienting (broken lines) with tone 512 as the orienting stimulus. Note that there are small bursts of deceleratory cardiac responses during orienting from trial 12 to trial 40 and again after 68 trials of orienting. During conditioning, solid lines, there are more cumulative responses than during orienting and the process is definitely more active. The inhibitory cardiac CR in this dog began to appear consistently after the 20th non-reinforced trial. The overall percentage of cumulated responses during orienting was 8% and during conditioning 33%.

The beat-to-beat HR analysis showed in some instances an initial sudden deceleration followed by an acceleration in HR two cardiac cycles after onset of the inhibitory CS, reaching a peak after the 4th beat; then the HR began to decline during the tone, decreasing below prestimulus HR level after the inhibitory CS. Averaging across, beat-to-beat rates, showed clear cut cardiac slowing after the inhibitory CS (see Figure 5).

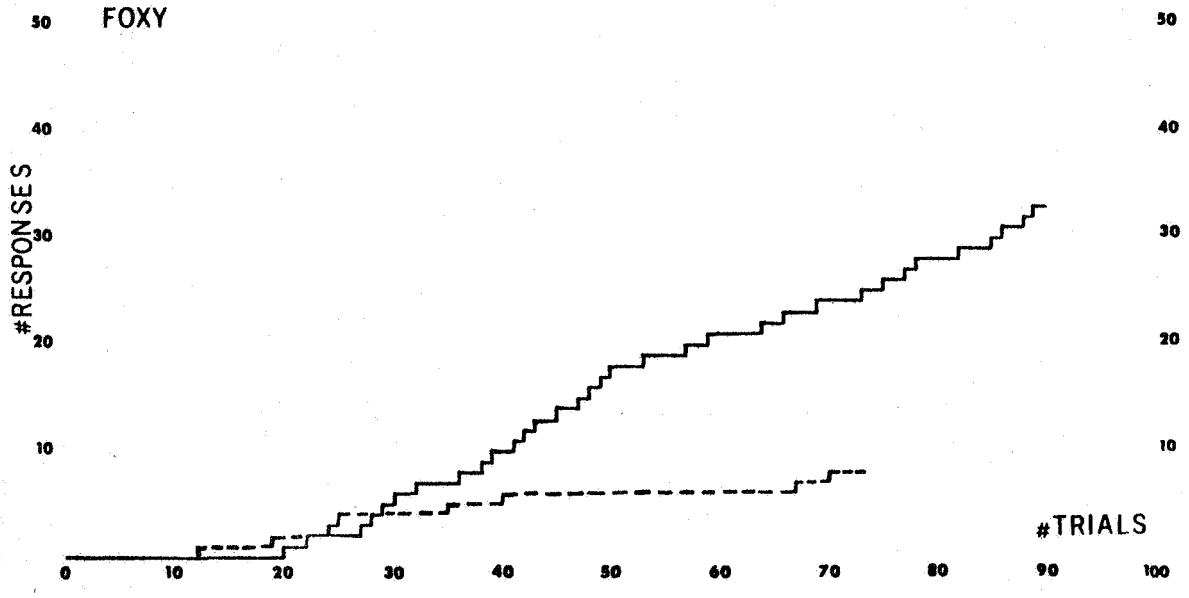


Figure 4: Tracing illustrating cardiac deceleration during orienting and inhibitory cardiac CRs during conditioning. Notice that the cardiac deceleration to the non-reinforced CS (-CS) is more active during conditioning than during orienting. See text for explanation.

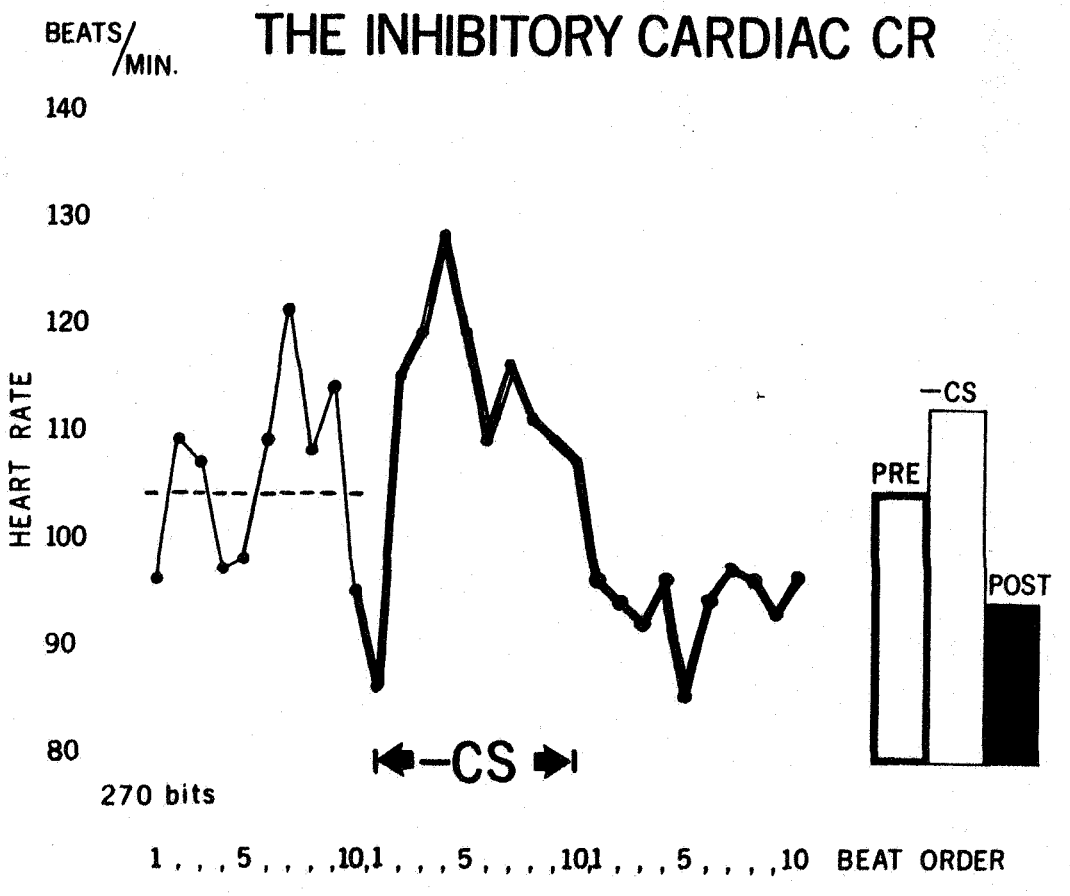


Figure 5: Average beat-to-beat analysis of five selected non-reinforced trials using a method described elsewhere (Newton & Perez-Cruet, 1965). Note that the pre-stimulus HR level (average across all prestimulus HR) is 105 bpm. During the -CS there is an initial sudden drop in HR to about 80 bpm followed by an acceleration to 128 bpm in the 4th R-R interval. After the peak acceleration there is a gradual decrease in HR to 84 bpm after the tone is off. Overall average of HR across R-R intervals is shown in the right corner of the figure, showing an average HR deceleration after -CS from 105 bpm (pre-stimulus) to 95 bpm after stimulus.

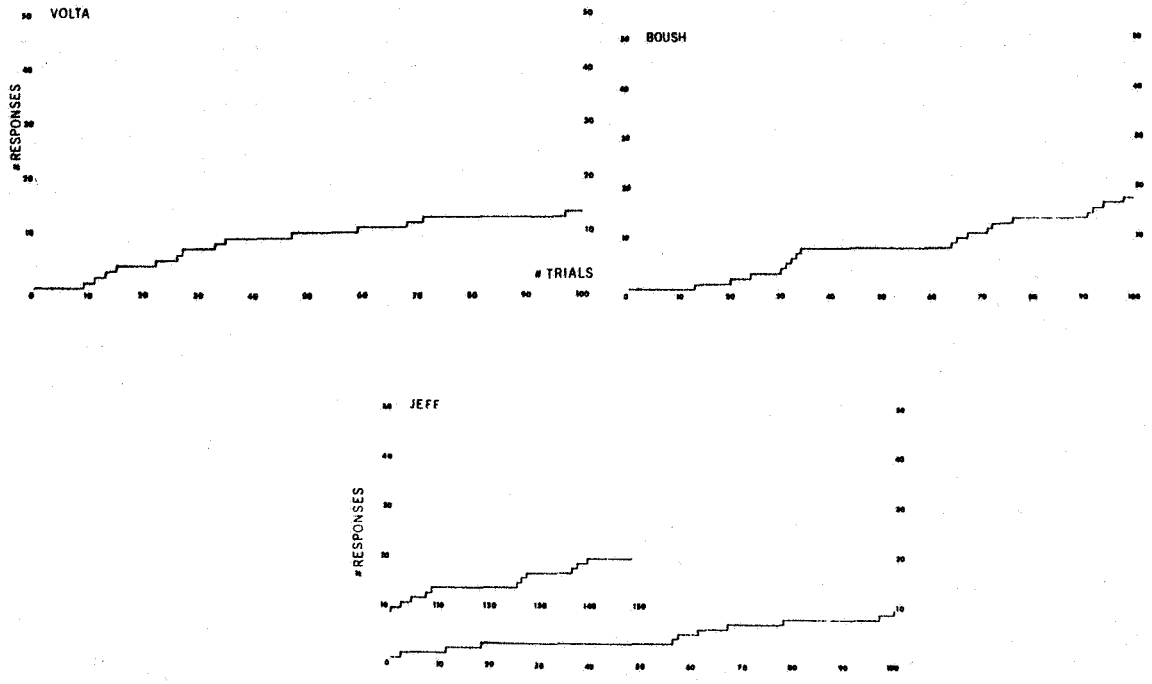


Figure 6: Cumulative tracing showing 3 dogs that did not show consistently inhibitory cardiac CR during -CS. Note that there are "bursts" of inhibitory cardiac CRs in Boush and Jeff. The dog, Volta, showed initially deceleration in HR to -CS but there process leveled after the 35th non-reinforced trial.

The results also showed that 30% of the dogs did not develop consistently inhibitory cardiac CRs as shown in Figure 6. In these dogs, bursts of inhibitory cardiac CRs were usually observed, especially in the dogs Boush and Jeff. The overall percentage of cumulative responses varied between 14% and 20% which was lower than in the dogs with active cardiac inhibition. These animals showed an acceleratory response or no response during the inhibitory CS.

Discussion:

The experiments show that the HR is a useful measure of inhibitory cardiac CR in classical conditioning. This finding supports Gantt's original hypothesis that cardiac functions can illustrate the establishment of active inhibition of decreasing below a baseline HR level (Gantt, 1960).

The HR measure indicated that inhibition was prominent in 70% of the dogs. In the remaining dogs that did not show HR deceleration during the non-reinforced trials (-CS) the inhibitory process was probably very weak. On the basis of our data, one can postulate that the inhibitory process represented by a HR deceleration does not occur in all animals. It is uncontroversial however, that the HR deceleration which occurs during or after the non-reinforced trials is an active process which in the Pavlovian paradigm represents active inhibition.

The use of cumulative tracings to illustrate active inhibition was more reliable than the overall percentages of the total responses although obviously percentages in groups of 10 trials would have also shown similar results to those in the cumulative record.

One has to seriously question whether all forms of cardiac decelerations are always inhibitory in nature. For example, the excitatory HR-CR in humans has been shown to be biphasic or deceleratory (Notterman, Schoenfeld & Bersh, 1952, Zeaman, Deane & Wagner, 1954, Wood & Obrist, 1964). Deceleratory HR-CR has been observed in cats (McLean, et al., 1956), rabbits (Kosupkin & Olmstead, 1943), rats (Bloch-Rojas, Toro & Pinto-Hamuy, 1964), opossums (Newton & Gantt, 1958) and occasionally in dogs (Gantt, 1953). Furthermore, deceleratory cardiac responses have been observed during expectancy situations in humans by Lacey (1964). Also HR deceleration has been observed during septal self-stimulation in behaviorally motivated animals (Malmo, 1961, Perez-Cruet, Black and Brady, 1963). The usefulness of the HR as an index of internal inhibition is applicable to the Pavlovian paradigm where the processes of excitation and inhibition are always competing, as Pavlov postulated originally with the salivary CR.

Studies by Fuhrer (1964) in humans have shown HR deceleration during non-reinforced inhibitory CS in experiments with controlled respiration. Fuhrer state that it "appears premature to invoke active inhibition as the physiological process which underlies the HR deceleration during inhibitory CS." Black, Carlson and Solomon (1962) have interpreted such a HR deceleration as an active inhibitory effect which supports Gantt's original hypothesis. We have applied this concept to cardiac functions with a knowledge of its limitations and usefulness in classical conditioning.

The use of cardiovascular functions as an index of Pavlovian inhibition is another measure which, taken in conjunction with other physiological parameters, may shed some light on the nature of the inhibitory process. The

application of the concept of internal inhibition in terms of HR deceleration is certainly valid in a Pavlovian paradigm. In explaining the inhibitory cardiac CR this concept is preferred because of the multiple physiological factors involved in the neural regulation of cardiac functions. Pavlov originally applied the term "braking process" (from Russian Tormoz) meaning inhibitory process. There is no doubt that such "braking process" is clearly illustrated in part B of Figure 1 and is represented by a gradual slowing of HR occurring during and/or after the inhibitory CS. Although the "braking process" is evident in individual trials, as well as during the development of differentiation, viz. discrimination, between excitatory and inhibitory CS, the physiological mechanisms underlying such a process are not clearly understood. In other words, it appears premature to invoke either parasympathetic or sympathetic mechanisms as the only mediators of the "braking process" but it is better at present to define such a process as inhibitory unless more psychophysiological mechanisms are demonstrated.

The concept of inhibition is perhaps one of the most important problems for study in the laboratory because it is here that perhaps some day science will be able to control and understand aggressive and destructive behavior in humans. These results indicate that the establishment of the inhibitory process can be studied using cardiac functions and in conjunction with other autonomic and behavioral parameters may be useful in elucidating the complex mechanisms responsible for the establishment of active inhibition.

Summary:

Inhibitory cardiac conditional reflexes were evaluated in terms of HR deceleration in 10 dogs. A Pavlovian paradigm was used with an excitatory tone (T 256) always reinforced with electric shock to the left foreleg and followed 2 minutes later by another tone (T 512) which was never reinforced. The HR deceleration during or immediately after the non-reinforced tone (-CS), viz. inhibitory cardiac conditional reflex, proved useful in detecting the spread of inhibition into cardiac functions after conditioning. The study supports Gantt's hypothesis that a change in HR which can fluctuate below a baseline may provide another measure of inhibition not available with the motor or salivary components where negative values are not possible.

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CARDIAC CONDITIONING IN DOGS WITH COMPLETE A-V BLOCK.

The purpose of this study is twofold: 1) to determine the effects of conditional stress in dogs with A-V block and 2) to determine the relative influence of psychic stimulation through conditional reflexes on atrial and ventricular rate. It is known that psychic stress produces detrimental effects on a diseased heart, such as the surgically blocked heart, but surprisingly there is no definite knowledge as to how these effects are mediated. Furthermore, there is no evidence available to us that chronic psychic stress will eventually lead to cardiac death. The fact that in the dog there is no definite efferent supply from the parasympathetic system to the ventricles, while the sympathetic supply to these chambers is abundant stimulated us to study the effects of a classical conditional reflex on atrial and ventricular rate in order to detect possible mechanisms through which the cardiac conditional reflex is mediated.

Five dogs have been used in this experiment. Surgical A-V block was produced by cutting the bundle of His with a technique described elsewhere by Starzl, Gaertner and Baker (1955). After surgical block the dogs were allowed to recuperate from surgery for 4 to 7 days. The animals were then isolated in a soundproof room and observed through a one-way window. The orienting reflex was studied for several days with techniques employed previously in our laboratory. The orienting training consisted of presentation of tones later used during conditioning, Tones 256 cycles per sec. and 512 cycles per sec. were presented alternately at 2 minute intervals for 5 to 20 trials per tone each day. The duration of the tone was 6 seconds. During the conditioning training, tone 256 cycles per sec. was always reinforced with a mild shock (5 to 10 volts) just sufficient to cause a withdrawal of the right foreleg. Tone 512 was never reinforced. The inter-trial interval varied between 1 to 2 minutes.

The techniques for evaluating atrial and ventricular rate changes were standard and it consisted of measuring the distance between p-waves for atrial rate and the distance between R waves for ventricular rate. Due to the fact that p-waves were occasionally buried in the QRS complex, atrial rate was measured in the R to R intervals where p to p intervals were clearly defined. In some cases when the p-waves were clearly defined within the QRS complexes they were used to measure p to p interval too.

The results have shown that after surgical A-V block the ventricular rate drops from control: 60 to 150 normal contractions per minute to 30 to 50 ventricular contractions per minute during complete A-V block. The degree of A-V block varied between 2:1 to 4:1 and atrio-ventricular dissociation was clearly evident. The atrial rates varied from 60 to 170 contractions per minute.

The motor orienting reflex, until extinguished, was accompanied by an increment in the atrial rate and a slight change in the ventricular rate. During conditioning, the cardiac conditional reflex was characterized by a prompt and significant rise in the atrial rate which varied from 130 to 190 atrial contractions per minute in Sally; 110 to 170 atrial contractions per minute in Mack; 150 to 169 contractions per minute in Temon; 170 to 220

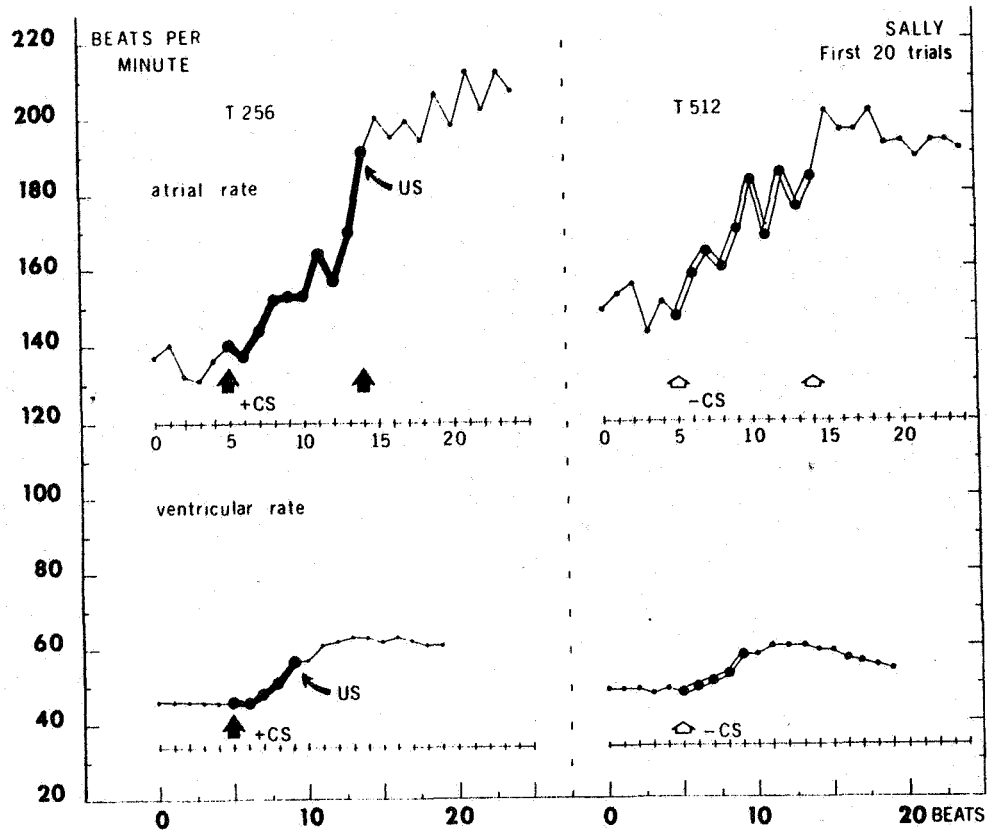


Figure 1. Illustrates atrial and ventricular rates before, during and after a reinforced (+CS) and non-reinforced (-CS) conditional stimulus. The upper half of the graph illustrates atrial rate to T 256 (+CS) and T 512 (-CS). Note that there is a significant increase in HR from 138 bpm to about 190 bpm during the presentation of the excitatory conditional stimulus. There is another acceleration in atrial rate produced by the unconditional stimulus (US) from 190 to 210 bpm. There is generalization of the conditional atrial reflex to T 512 which illustrates lack of differentiation in the early part of conditioning. The ventricular rate is shown in the lower part of the figure. Note that during the +CS there is a conditional increase in the ventricular rate from 43 bpm to about 58 bpm. Note also that conditional ventricular reflex is generalized to the -CS. The unconditional stimulus (US) produces an acceleration in ventricular rate of about 5 beats. The abscissa is the consecutive beats counted and the ordinate represents the cardiac rate for atrial and ventricular contractions. The ventricular rate was measured successively, whereas the atrial rate was measured consecutively as described in the text.

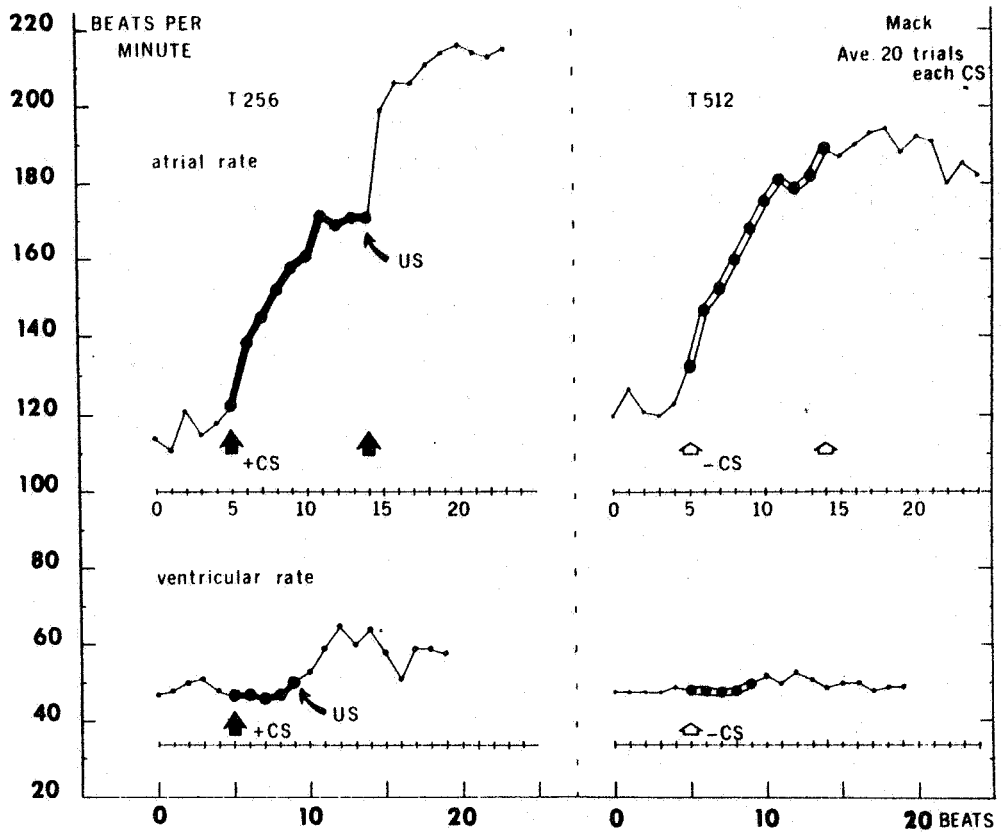


Figure 2. Illustrates atrial and ventricular rates before, during and after a reinforced (+CS) and non-reinforced (-CS) conditional stimulus. The upper half of the graph illustrates atrial rate to T 256 (+CS) and T 512 (-CS). Note that there is a significant increase in HR from 120 bpm to about 170 bpm during the presentation of the excitatory conditional stimulus. There is another acceleration in atrial rate produced by the unconditional stimulus (US) from 170 to 218 bpm. There is generalization of the conditional atrial reflex to T 512 which illustrates lack of differentiation in the early part of conditioning. The ventricular rate is shown in the lower part of the figure. Note that there is no significant change in the ventricular rate during the conditional stimulus either during the +CS or the -CS. The unconditional stimulus (US) produces an acceleration in ventricular rate of about 10 beats. The abscissa is the consecutive beats counted and the ordinate represents the cardiac rate for atrial and ventricular contractions. The ventricular rate was measured successively, whereas the atrial rate was measured consecutively as described in the text.

atrial contractions per minute in Gus Gus; and 78 to 118 atrial contractions per minute in Dumbell. Three dogs, Sally, Gus Gus and Dumbell, showed increments in ventricular rate (3 to 10 beats per minute) as a cardiac conditional reflex. The other two dogs showed no significant change in the ventricular rate.

Figure 1 illustrates changes in ventricular and atrial rate during a reinforced tone (T 256) and a non-reinforced tone (T 512) in the first 20 trials of the conditioning training. Note that there are definite increments in the atrial rate to T 256 (+CS) and T 512 (-CS) showing generalization of the conditional reflex to the inhibitory non-reinforced tone early in conditioning. Note also that the marked atrial rate changes are accompanied by a slight increase in the ventricular rate. The atrial rate is responding faster and earlier during the conditional stimulus than does the ventricular rate. The unconditional stimulus (US), or shock to the foreleg produced a further increase in atrial and ventricular rates.

Figure 2 illustrates a conditional reflex of the atrial rate. In this dog, there is a definite conditional atrial acceleration to tones 256 and 512 during early conditioning (first 20 trials). However, in spite of this marked conditional atrial acceleration there was no clear evidence of conditional ventricular acceleration. The unconditional stimulus (US), as in the previous dog, produced a definite unconditional increase in atrial and ventricular rates.

The results show that the conditional atrial reflex is definitely stronger than the conditional ventricular reflex. The fact that three dogs developed conditional ventricular rate changes indicates that sympathetic nervous impulses to the ventricles are activated during the conditional process. The data show clearly that the main influence on the heart is through the sino-auricular node because all dogs showed conditional atrial changes.

The delayed conditional ventricular reflex suggest: 1) that this response is probably mediated through neurohumoral mechanisms; 2) that the spread of conditional excitation has to be very strong in order to influence the ventricles; and 3) that the conditional excitation of the ventricles is slower than the conditional atrial excitation.

None of the dogs developed heart failure during the conditioning training, and two of the animals are still alive 2 and 3 years after surgery. Three of the animals were sacrificed and they all showed enlarged hearts as reported previously by Starzl and Gaertner (1955).

In summary, the study indicates that conditional stress produced temporary stimulus-bound changes in atrial and ventricular rates with no precipitation of cardiac death. The psychic influence through conditioning on the A-V blocked heart is dual that is, through the sino-auricular pacemaker and through ventricular pacemakers, but the stronger pathway for such an influence appears to be through the sino-auricular node. Since the normal pathway between the sino-auricular pacemaker and the ventricle was cut this allowed us to determine the activation of ventricular pacemakers to psychic stimulation. The study clearly shows that the conditional psychic reflex activates the ventricles as well as the sino-auricular pacemaker.

EFFECTS OF ALPHA, BETA ADRENERGIC AND PARASYMPATHETIC BLOCKADE ON CARDIOVASCULAR CONDITIONING.

The purpose of this study was to determine the role played by alpha and beta blockade in dogs with chronic cervical vagotomies. Our experiments were performed in unanesthetized dogs that had well established cardiac conditional reflexes. Alpha adrenergic blockade was done with dibenzyline; and beta adrenergic blockade was done with dichloroisoproterenol (DCI) or propranolol (Inderal). In order to isolate the cardiac beta receptor mechanisms, some dogs were bilaterally vagotomized or pretreated with atropine. No vagotomy or atropine pretreatment was done in the alpha adrenergic receptor study because as far as we know, alpha receptors are not present in the heart. In 7 dogs we have studied heart rate and blood pressure either under the alpha or beta receptor blockade. In 2 of these we have also studied other hemodynamic parameters such as aortic blood flow and ventricular pressures.

The results show that alpha receptor blockade influenced the cardiac conditional reflexes in some dogs, i.e. dibenzyline diminished slightly the HR-CR either early after injection of dibenzyline or 30 minutes later, but in some dogs these results were not consistent. Dibenzyline blocked the BP-CRs completely. Dibenzyline given to a dog with a bradycardia HR-CR showed inversion of the HR-CR to a tachycardia HR-CR.

The results with beta adrenergic receptor blockade were more conclusive. DCI usually blocked the HR-CRs, but DCI had an undesirable sympathomimetic side effect which consisted of an increase in the HR baseline from 120 to 170 bpm. The more specific beta adrenergic receptor blocking agent, propranolol (Inderal), did not have undesirable sympathomimetic effects and it usually produced a decrease or no change in the baseline HR level. Occasionally a slight increase in the baseline HR was observed in some dogs. Propranolol alone without vagotomy or atropine pretreatment could not completely block the cardiac conditional reflexes although they were diminished after the drug. The dosage required to obtain this partial diminution of HR-CR varied between 5 to 10 mg/Kg., I.V.. In the dogs that had been pretreated with atropine or bilateral vagotomy, propranolol in doses from 1 to 5 mg/Kg. obliterated completely the HR-CRs.

In 5 dogs in which we observed BP-CRs, propranolol blocked or changed the magnitude of the BP response in 4 of the 5 dogs. In a dog in which propranolol did not block the BP-CR, pretreatment with succinylcholine had been carried out and this could have contributed to this effect. This might suggest that propranolol had blocked some peripheral beta adrenergic mechanisms which could have influenced the BP-CR.

The results with alpha and beta adrenergic blocking agents indicates that in the normal animal, where parasympathetic and sympathetic innervation is intact, these two systems are constantly interacting. That is, if one blocks only the beta and not the parasympathetic mechanism one can still observe an acceleratory or deceleratory HR-CR. Similarly if one blocks only the parasympathetic either with atropine or surgical vagotomy one can still get an acceleratory HR-CR which is mediated by the sympathetic nervous system, and one has isolated the beta receptor influence. When this is accomplished propranolol or DCI will block completely the mediation of the HR-CR. This fact indicates that the cardiac conditional reflex is a neural event and that it is not mediated by peripheral factors.

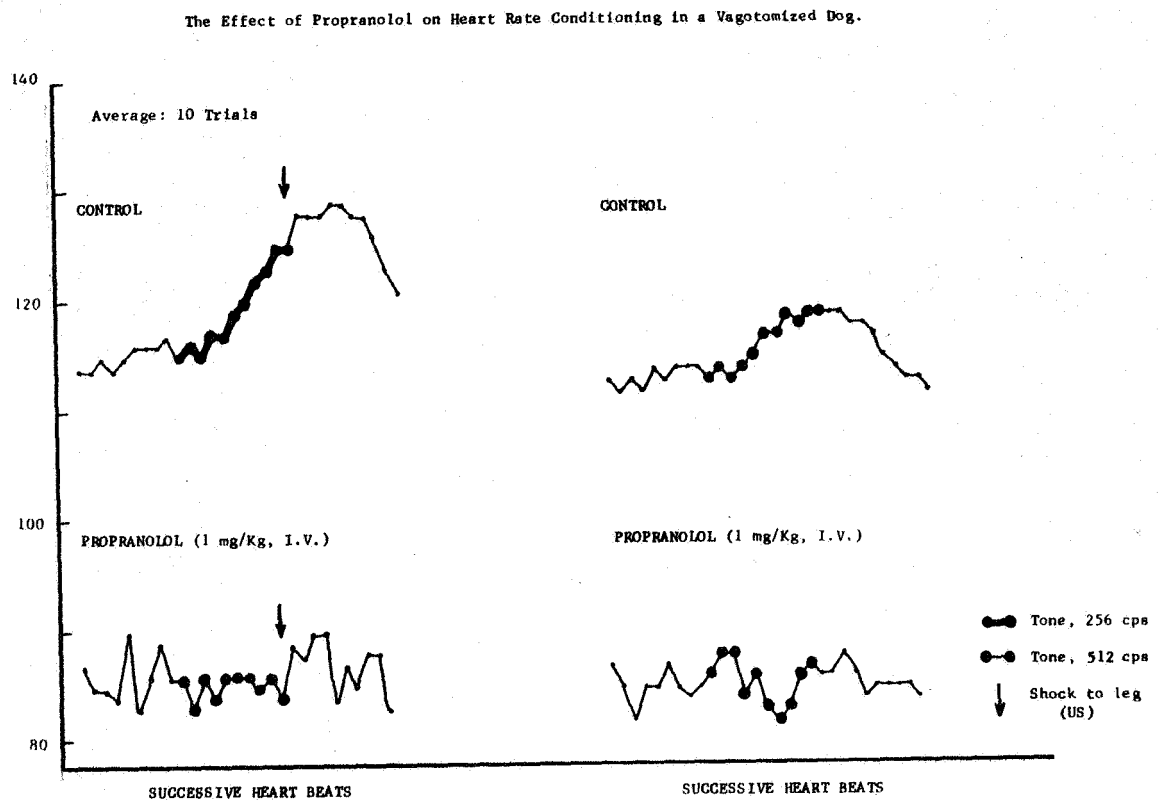
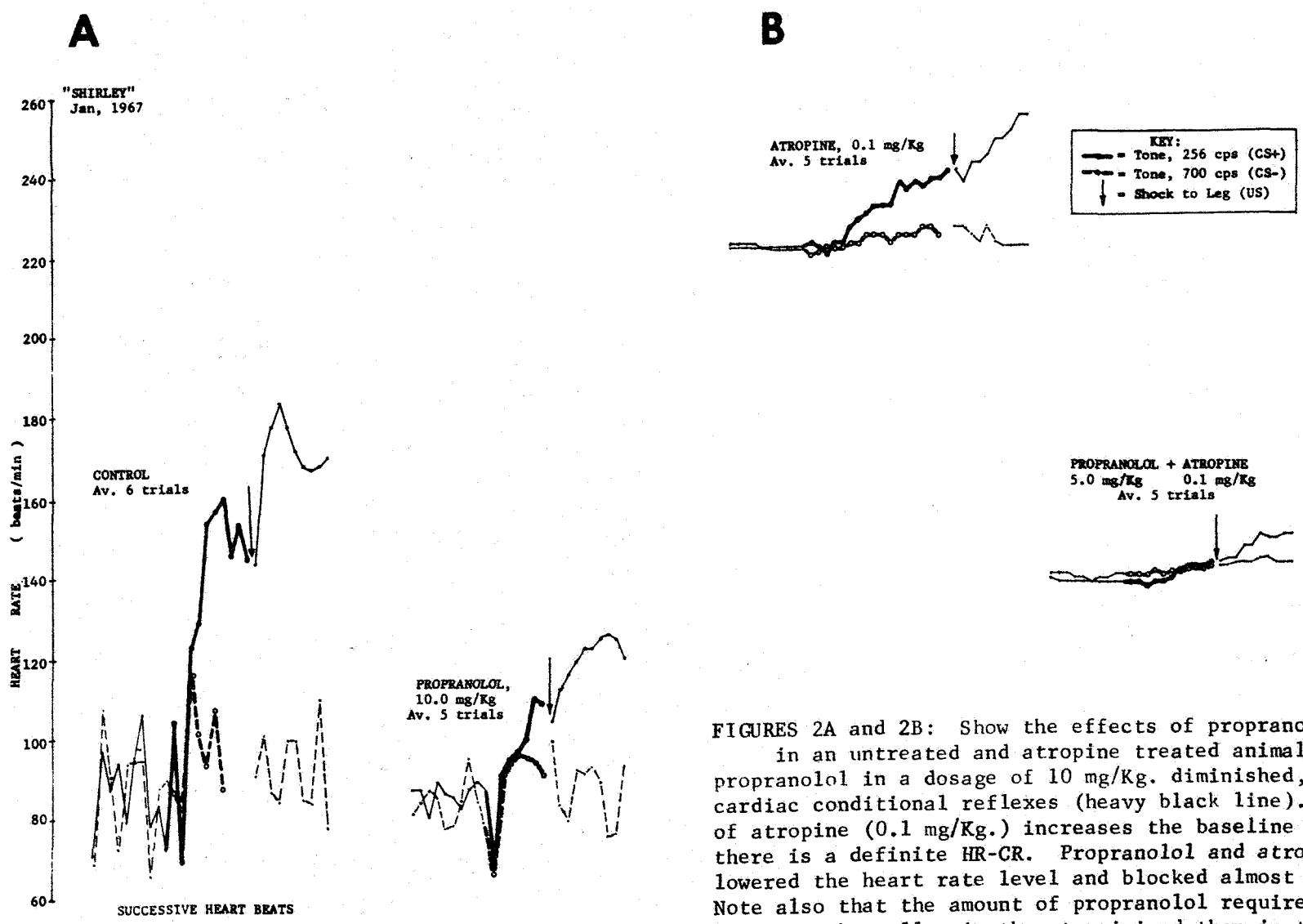


FIGURE 1: Illustrates the effects of propranolol on cardiac conditioning in a vagotomized dog. Upper control tracings show conditional heart rate changes to the reinforced CS (T256 cps) and non-reinforced CS (T512 cps). Note that there is a significant ($p < 0.001$) increase in heart rate to T256 and some generalization to T512 cps. Propranolol (lower two curves) in doses of 1 mg/Kg., I.V. blocked completely the conditional cardiac responses in vagotomized dogs. It is of interest to point out that the propranolol also decreased significantly the effects produced by the painful stimulation. Propranolol also lowered the baseline HR level in the chronically vagotomized dog.



FIGURES 2A and 2B: Show the effects of propranolol on cardiac conditioning in an untreated and atropine treated animal. Note that in part A propranolol in a dosage of 10 mg/Kg. diminished, but did not block, the cardiac conditional reflexes (heavy black line). In part B an injection of atropine (0.1 mg/Kg.) increases the baseline heart rate level and there is a definite HR-CR. Propranolol and atropine in the same experiment lowered the heart rate level and blocked almost completely the HR-CR. Note also that the amount of propranolol required to block the cardiac CRs is much smaller in the atropinized than in the untreated animal at A.

CARDIAC CONDITIONING IN DOGS WITH CHRONIC BILATERAL CERVICAL VAGOTOMIES.

The purpose of this study was to determine if cardiac conditioning (HR-CR) could be established in dogs with chronic bilateral cervical vagotomies. The experiments were conducted in five dogs in which the vagi nerves were resected in the neck at the level of the 3rd cervical vertebra and which had had corrective surgery for pylorospasm, achalasia and vocal cord paralysis. All dogs had an initial training in which tones of frequencies 256 and 512 cycles per sec. were presented before conditioning to determine the cardiac orienting reflex (HR-OR). After the initial evaluation of the HR-OR, to the above tones, classical conditioning training was started with an excitatory tone (T256 cps) always reinforced with a mild electric shock to a foreleg followed two minutes later by another tone of frequency 512 cps which was never reinforced. The duration of these tones was six seconds. Experiments were conducted in a soundproof room for periods ranging from 2 to 8 weeks. The results showed formation of cardiac conditioning in 3 out of 5 dogs. The baseline heart rate (HR) in the vagotomized dogs was 135, 125, 250, 150 and 134 beats per minute (bpm) respectively. During orienting there were small increments in HR from 3 to 10 beats above baseline as a HR-OR. The HR-OR consisted of a marked acceleration in HR from 135 to 155; 150 to 174; and 130 to 158 bpm respectively during the excitatory tones in the three dogs that showed cardiac conditioning. Figure 1 illustrates a HR-CR and BP-CR to the reinforced conditional stimulus in a dog with bilateral cervical vagotomy. The unconditional response to the electric shock, namely the HR-UR, varied between 10 to 55 bpm above baseline HR. Heart rate differentiation between tones 256 and 512 cps was observed in two dogs. Figures 2 and 3 illustrate differentiation in HR-CR between a reinforced (T256) and a non-reinforced (T512) tone, respectively. The latency of the HR-CR was usually 3 to 7 heart cycles. During classical conditioning training all dogs showed motor defensive responses to the electric shock. Strong motor conditional reflexes were observed in two dogs which also showed motor differentiation. The others showed weak motor conditioning. The present study indicates that cardiac conditioning can be established in vagotomized dogs, provided the dogs are maintained in healthy condition. This study supports preliminary data from our laboratory which have suggested that the sympathetic system plays an important role in the establishment of cardiac conditioning.

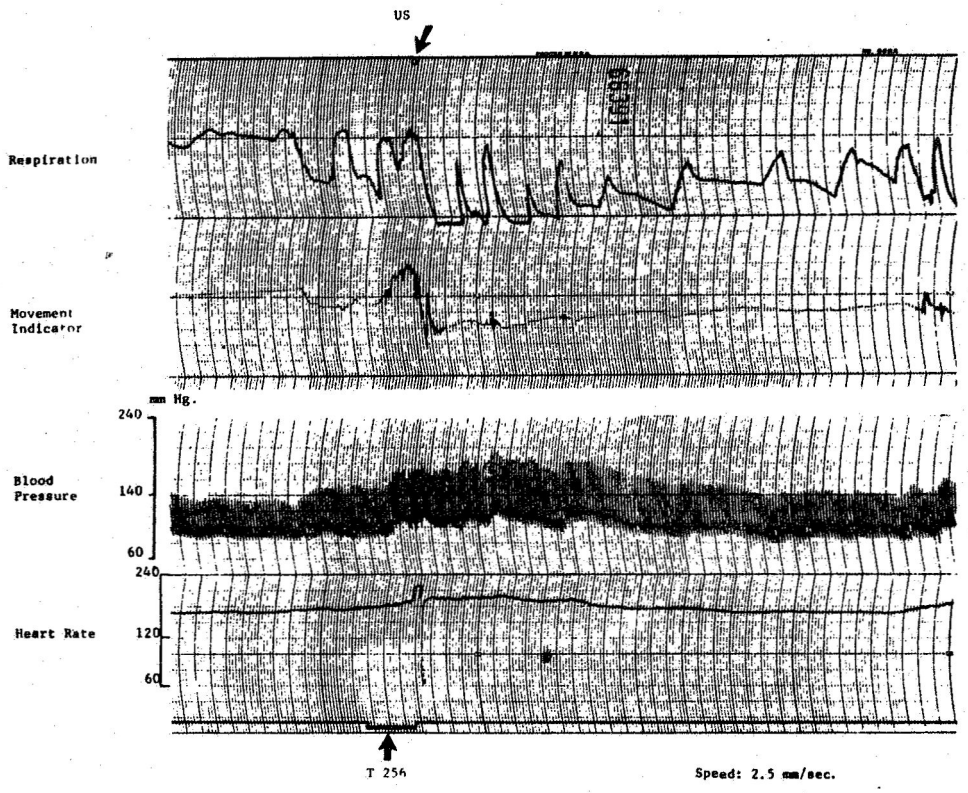


Figure 1. Illustrates Respiratory, Motor, Blood Pressure and Heart Rate conditional reflexes in a dog (Wallace) with chronic bilateral cervical vagotomy. Note that the Movement Indicator shows anticipatory movements before the excitatory tone followed by a footlift during the tone. Motor conditional reflexes, shown by the Movement Indicator, is accompanied by conditional changes in heart rate, blood pressure and respiration. See the marker for T256. The unconditional painful electric stimulus (US) to the skin, indicated at the upper part of the tracing by the arrow, produces a further withdrawal of the leg and changes in blood pressure, respiration and heart rate as unconditional reflexes.

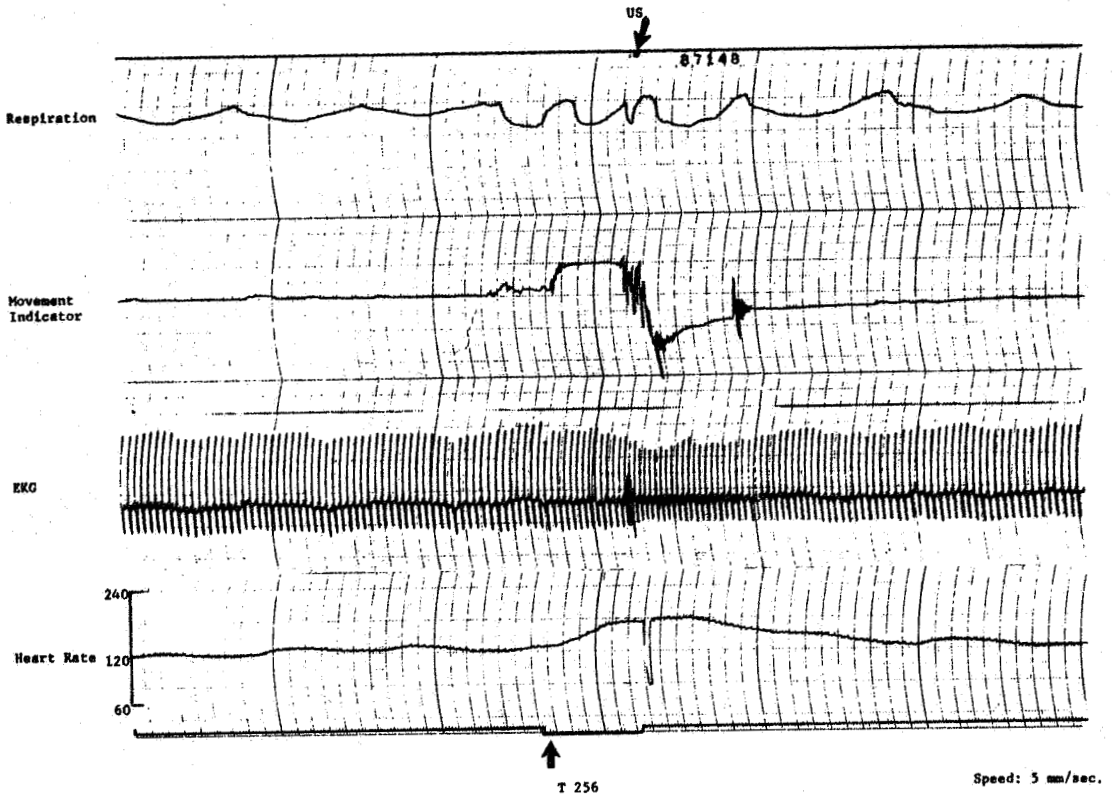


Figure 2. Illustrates Respiratory, Motor and Heart Rate conditioning to the excitatory tone in another dog (Chocolate) after 69 trials. Note that during the tone the respiratory rate increases. There is a footlift as shown by the Movement Indicator and there is a definite increase in heart rate during the tone. The unconditional stimulus (US), marked with the upper arrow, produces an increase in respiration, movement of the leg and a heart rate change.

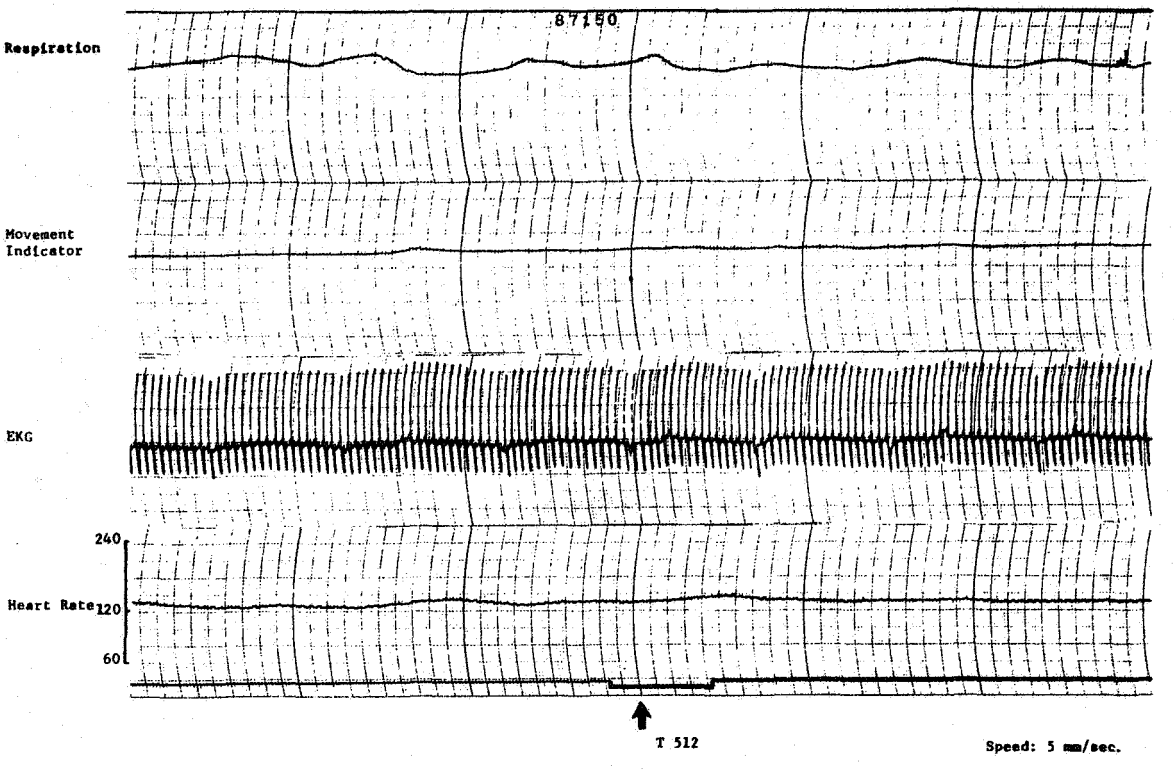


Figure 3. Illustrates differentiation of conditional reflexes in Chocolate. Note that there is a very slight change in the respiratory rate, no movements and no change in heart rate when the non-reinforced T512 was presented. The tracing in Figure 3 was obtained 2 minutes after the presentation of the 69th reinforced trial with T256 shown in Figure 2.

CARDIOVASCULAR CHANGES DURING FIRST TRIALS OR ORIENTING IN DOGS WITH BILATERAL CERVICAL VAGOTOMIES.

A recent review on heart rate (HR) changes as a component of the orienting responses (Graham & Clifton, 1966) has shown that numerous authors have reported an initial deceleration in HR during the early phases of orienting. According to Sokolov this initial slowing of heart rate is a true orienting response which might be associated with attention and focusing reactions. Recent reports by Lynch, 1967, show that 90% of the dogs studied by him showed definite deceleration of HR during the first 10 trials of orienting. Prior to Lynch's study this laboratory, Robinson and Gantt, 1947, reported both acceleration and deceleration in HR during early orienting. The early orienting bradycardia was considered to be a vagal phenomenon.

The purpose of this study was to determine if the initial orienting bradycardia could be observed in dogs in whom the vagosympathetic trunks had been cut bilaterally at the level of the second and third vertebrae. Since the HR deceleration in early orienting has been implicated as being a vagal origin, we would have expected that a bilateral cervical vagotomy would completely obliterate this response.

A total of 5 dogs have been employed in these studies which are still in progress. In 3 dogs we have observed a definite bradycardia during the first orienting trials. The bradycardia during early orienting in the vagotomized dogs is not as prominent as in the normal dogs. Recording of blood pressure simultaneously with the HR orienting reflexes has ruled out that the bradycardia is of baroreceptor origin because during the slowing of HR in the first trials of orienting the systolic and diastolic blood pressure is also decreased. The respiratory rate during early orienting has also decreased, but there have been cases in which deceleration occurs independent of respiration.

Studies are now in progress to determine if the initial bradycardia observed in these animals is mediated solely by the sympathetic nervous system or by possible hemodynamic changes. Blockade with a beta adrenergic receptor blocking agent (propranolol) will allow us to rule out hemodynamic factors and will enable us to determine the sympathetic mediation of this orienting bradycardia response.

VAGO-RENAL REFLEXES ASSOCIATED WITH AFFERENT VAGAL STIMULATION.

Renal secretion was measured in dogs with chronic implants of an ureter catheter before, during and after afferent stimulation of the left vagosympathetic trunk in a preparation where the right vagosympathetic trunk has been cut. Stimulation of the left vagosympathetic trunk still connected to the medullary centers but disconnected distally to the viscera, produced a mild diuretic reflex. The latency of the diuretic reflex was delayed and it was evident 30 to 45 seconds after afferent stimulation in two dogs. The mediation of this diuretic reflex is probably carried by afferent fibers in the vagosympathetic trunk and it is probably related to Henry-Gauer's auriculo-renal reflex which consists of a renal diuresis when the right auricle is distended. This preliminary data suggests that we are probably stimulating afferent fibers which could mediate the Henry-Gauer diuretic reflex.

CHANGES IN HEART RATE, BLOOD PRESSURE AND RESPIRATION DURING AFFERENT VAGAL STIMULATION.

Rosenblueth and Freeman (1931) reported a deceleration in heart rate (HR) by afferent stimulation of the vagosympathetic trunk. Their studies suggest that afferent stimulation in some instances produced a deceleration of HR. On the basis of this observation they concluded that there were cardiac inhibitory functions carried through sympathetic nerves and their experiments also suggested possible connections between sympathetic and parasympathetic medullary nervous pathways.

The present was designed to determine the changes in HR, respiration and blood pressure (BP) produced by afferent vagal stimulation in anesthetized and unanesthetized animals. Four dogs have been used in this study. The animals were anesthetized with pentobarbital anesthesia. Afferent biphasic stimulation, at a 30 cycles/second, 2 to 3 volts intensity, ½ to 1 second duration, was done first through the left vagal sympathetic trunk which had been cut distally but with the right vagosympathetic trunk intact. After obtaining the effects of the afferent stimulation with right vagus intact, the right vagosympathetic trunk was subsequently cut allowing only true afferent vagal stimulation. The results obtained by stimulation of the left vagosympathetic trunk with intact right vagosympathetic were similar to those obtained by Andrus, et al., 1966. Afferent stimulation of the left vagosympathetic trunk were the right vagosympathetic trunk cut revealed in about 90% of the trials a definite, but less marked, deceleration in HR. There was an inhibition of respiration during afferent vagal stimulation. The BP changes showed an increase in systolic and diastolic BP in one animal during and after afferent stimulation of the vagosympathetic trunk; three other animals showed slight decreases in BPs accompanying the slowing of HR.

The results in four animals showed that afferent stimulation of the left vagosympathetic trunk in a preparation in which the right vagosympathetic trunk has also been cut, namely a true afferent stimulation, showed a slowing of HR and inhibition of respiration. The results confirmed Rosenblueth and Freeman's work and they also showed that inhibitory functions on HR can be mediated through the sympathetic system. This fact was confirmed by giving an adrenergic blocking agent (propranolol) which completely obliterated the responses elicited by afferent vagal stimulation.

The fact that this slowing in HR is mediated by the sympathetic nervous system was confirmed using pharmacological blockade of the beta adrenergic receptor mechanisms. After beta receptor blockade the HR slowing produced by afferent stimulation was abolished.

THE EFFECT OF PERSON ON HEART RATE AND BLOOD PRESSURE IN MONKEYS.

Previous investigations from our laboratory have shown that the presence of a person can induce marked heart-rate changes in dogs. Previously, we have shown marked changes in heart rate (HR) in monkeys produced by a person entering an experimental room.[1] This response is probably mediated through the sympathetic nervous system.

The response to a person can be divided into two main components: one, the initial response to a person which acts as a primary stimulus and two, the exit or removal of the person-stimulus component. The response of primates to the entrance of a person into the room is very complex, and it involves the musculo-skeletal and autonomic systems. The psychocardiovascular responses usually consist of an acceleration in HR. It has been shown that this initial response is different from tactile interactions such as petting an animal in which the psychocardiovascular response usually consists of a deceleration in heart rate[2]. The exit component or removal of the person-stimulus usually but not invariably produced a deceleration in HR.

The purpose of this study was twofold: one, to determine the effect of person on blood pressure (BP) and HR and two, to investigate some of the mechanisms through which this response is mediated.

Methods and Materials

Eight rhesus monkeys (Ss), weighing from 10 to 14 lbs., were studied. In 4 Ss, only HR was recorded. In the other 4Ss, direct BP and HR were recorded simultaneously using techniques described elsewhere [3,4]. All Ss were restrained in monkey chairs and isolated in a soundproof room. Physiological measurements were recorded on a Type R Offner polygraph.

Results

The results showed marked changes in BP and HR when the person entered the soundproof room, as shown in Figure 1. Pre-stimulus HR levels in 4 monkeys were: 150, 144, 108 and 129 bpm respectively. The entrance of a person into the experimental room increased the HR from 6 to 13 heart beats above pre-stimulus levels in the first 10 R-R beat-count after entering the room. The next 10 R-R beat-count showed an increase of 9 to 24 heart beats above pre-stimulus levels. The acceleration of HR produced by a person entering the room was highly significant ($p < .001$). Figure 2 illustrates the beat-by-beat analysis of HR changes in 4 monkeys when a person enters the room. Pre-stimulus BP values in 4 monkeys were: 156/96, 104/66, 158/92 and 160/92 respectively. The entrance of a person into the experimental room increased the BP to 178/122, 122/76, 168/102 and 190/110 respectively. The latencies of the BP response varied between 3 to 21 seconds.

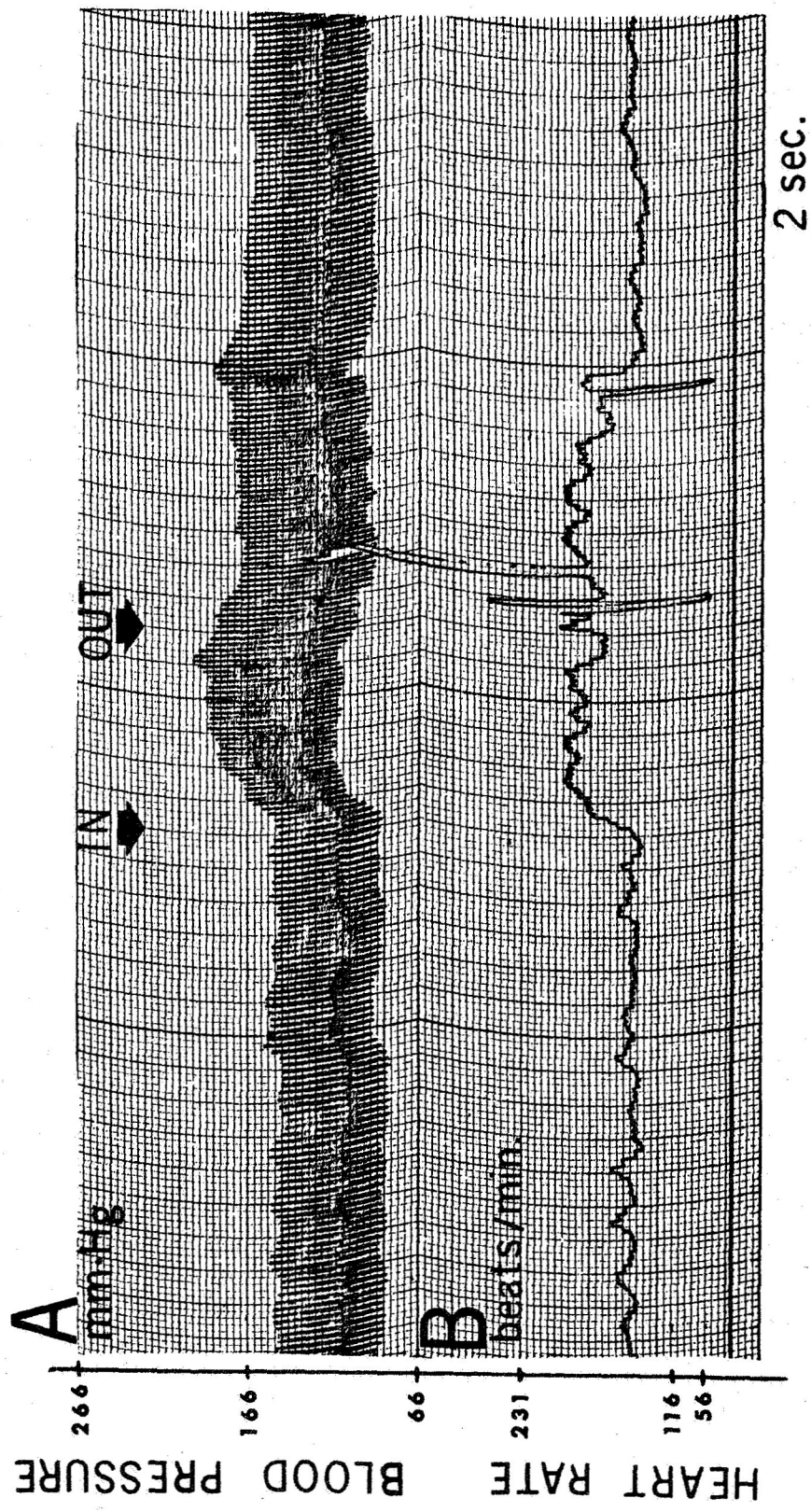

A
mm.Hg
B
beats/min.
IN
OUT
2 sec.
266
166
66
231
116
56
BLOOD PRESSURE
HEART RATE

FIGURE 1

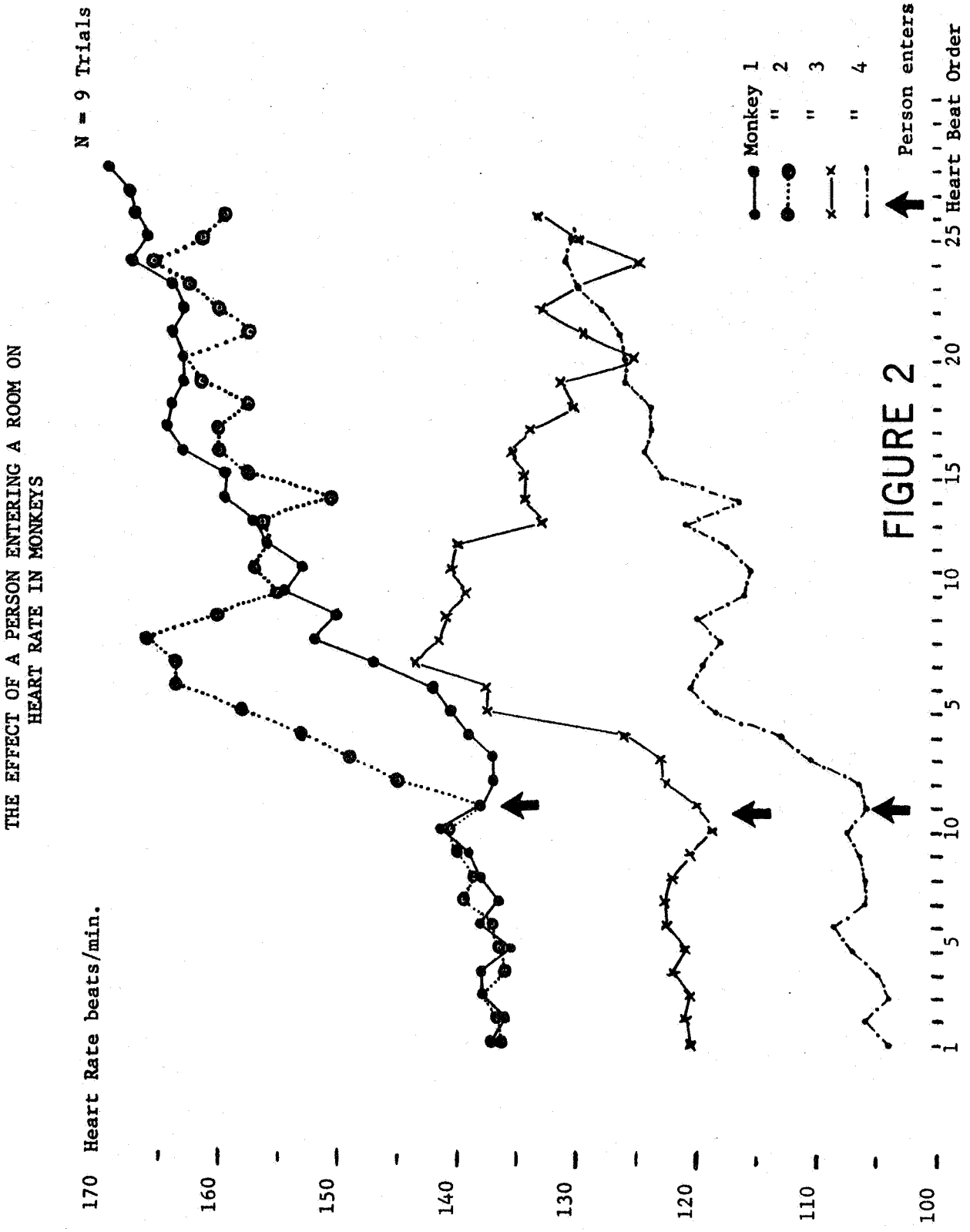

THE EFFECT OF A PERSON ENTERING A ROOM ON
HEART RATE IN MONKEYS
N = 9 Trials
170 Heart Rate beats/min.
160
150
140
130
120
110
100
1
5
10
15
20
25 Heart Beat Order
Monkey 1
" 2
" 3
" 4
Person enters room

FIGURE 2

TABLE 1

EFFECT OF ATROPINE AND DIBENZYLINE ON THE BLOOD-PRESSURE RESPONSE TO A PERSON ENTERING A ROOM.

	N	Pre-stimulus Blood Pressure*	Blood-Pressure Response to Person
Pre-Drug	39	S** 148 D** 92	S 168 D 105
Atropine (SQ-0.1 mg/kg)	28	S 155 D 100	S 174 D 110
Pre-Drug	39	S 148 D 92	S 168 D 105
Dibenzyline (IV-10 mg/kg)	11	S 66 D 30	S 66 D 30

* Blood pressure in mm. Hg measured intra-arterially with a technique described elsewhere[3].

** Systolic ** Diastolic

Drug Studies

Drug studies were employed in order to elucidate the mechanisms involved in the mediation of the psychocardiovascular response to person. Atropine injected in dosages of .1 mg.kg in 1 monkey did not block this response. However, the response was blocked completely with dibenzyline in intravenous dosages of 10 mg/kg. Table 1 summarizes the results with drugs. That the response to a person is mediated via sympathetic pathways is supported by experiments now in progress, because it has been observed in animals with chronic mid-cervical vagatomies. Further evidence that this response is sympathetic in origin has been obtained in dogs with surgical AV block in which the ventricular rate is increased through the effect of person.

Summary

This preliminary data suggests that the psychophysiological mechanisms underlying the effect of person might be important in assessing sympathetic influences on the cardiovascular system. The experiment might also be useful in elucidating the mechanisms involved in the interaction between a person and an experimental subject.

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CHANGES IN HEART RATE AND BLOOD PRESSURE DURING VESTIBULAR STIMULATION USING CALORIC TESTS.

Cardiovascular changes in humans during caloric stimulation of the vestibular apparatus are well known. Measurement of these cardiovascular functions in monkeys was undertaken in order to quantify the degree, nature and direction of cardiovascular changes during severe nystagmus following caloric stimulation. If there were significant cardiovascular changes following caloric stimulation, cardiac conditioning would be attempted. Four rhesus monkeys were studied. Heart rate was obtained using implanted S.Q. electrodes; blood pressure was measured using a chronic intra-arterial indwelling catheter; eye movements were measured with implanted electrodes on each side of the orbit of the eye. Tests were made in anesthetized (Sernylan 1 to 2 cc S.Q.) and in awake monkeys. The awake monkeys were restrained in Foringer chairs. Hot or cold water, for caloric stimulation of the vestibular apparatus, was introduced into the external auditory canal through a long catheter which had been implanted to facilitate chronic irrigation of the ear.

Heart rate and blood pressure changes were measured only when the monkeys had unmistakable nystagmus after caloric stimulation. The cardiovascular changes associated with nystagmus were evaluated by comparing the average of 10 consecutive blood pressure readings, systolic and diastolic, and heart rate recordings, i.e. a period of 10 heart beats of the period just before and after irrigation began and a period during severe nystagmus.

Out of a total of 68 tests only 25 precipitated a clear-cut episode of horizontal nystagmus which was also clearly recorded with the implanted electrodes. During "anesthesia" with Sernylan*, 15 clear-cut episodes of horizontal nystagmus were induced. In awake animals precipitation and stimulation of nystagmus was more difficult probably due to spatial orientation or compensatory mechanisms. In the "anesthetized" monkeys, severe nystagmus produced a slight increase in average systolic pressure (4.7 mm Hg.) and average diastolic pressure (6.2 mm Hg.). In the awake monkeys, similar changes in blood pressure were observed (average systolic pressure increased 4.9 mm Hg. and diastolic pressure 5 mm Hg.). In "anesthetized" animals the average heart rate changes associated with nystagmus were also very minimal (6.0 beats per minute in the awake animals).

Tables 1, 2 and 3 show the results of individual tests before and after caloric stimulation; before and after beginning of nystagmus; during severe nystagmus; and after nystagmus. Direct blood pressure, systolic and diastolic determinations, are shown on the left of tables (A). Heart rate changes, shown on the right of tables (B), were measured before and during nystagmus. The results of our experiments showed inconsistent cardiovascular changes during severe nystagmus induced with caloric stimulation by irrigation of the external auditory canal. However, hot irrigation produced increases in blood pressure more consistently than cold water irrigation.

The experiments were attempted to determine the conditionability of vestibular responses in primates, but due to the inconsistency of the response no further conditioning was attempted.

TABLE 1

MONKEY	DATE	A IRRIGATION BEFORE	A IRRIGATION AFTER	NYSTAGMUS BEGINS BEFORE	NYSTAGMUS BEGINS AFTER	SEVERE NYSTAGMUS DURING	NYSTAGMUS ENDS	B HEART RATE CONTROL	B HEART RATE NYSTAGMUS	REMARKS
215	7/21/66	S:135.3*	133.9	152.5	152.4	150.8	144.2	186	192	R-H
		D: 79.3	87.3	102.4	101.6	101.2	96.1			
"Anesthetized"										
		S:139.3	144.4	129.8	130.5	127.6	120.1	198	204	L-H
		D: 93.6	98.5	83.3	81.1	79.5	74.3			
		S:133.9	132.6	158.2	146.8	157.9	151.9	186	222	R-H
		D: 90.3	90.4	115.8	103.5	112.5	107.5			
		S:128.2	133.1	137.6	139.7	152.9	141.2	198	210	L-H
		D: 86.2	92.6	97.4	99.9	108.5	97.9			
		S:129.9	128.1	125.5	125.2	137.0	129.3	186	198	R-C
		D: 90.0	87.1	86.4	85.7	94.4	87.8			
		S:130.1	128.9	132.8	132.7	156.8	142.5	192	204	L-C
		D: 84.1	79.7	86.8	85.0	106.7	96.3			
		S:133.3	131.4	127.8	128.7	126.5	125.4	204	204	R-C
		D: 87.9	85.8	82.0	81.8	83.4	78.9			
		S:120.2	117.5	121.9	127.2	128.0	130.9	204	216	L-C
		D: 73.6	72.1	75.2	82.3	84.4	85.2			

* Average of 10 beats

** Route: R-H = right ear, Hot H_2O
L-H = left " " "
R-C = right ear, Cold H_2O
L-C = left " " "

S = Systolic Blood Pressure
D = Diastolic " "

TABLE 2

MONKEY	DATE	A IRRIGATION BEFORE	IRRIGATION AFTER	NYSTAGMUS BEGINS BEFORE	NYSTAGMUS BEGINS AFTER	SEVERE NYSTAGMUS DURING	NYSTAGMUS ENDS	B HEART RATE CONTROL	HEART RATE NYSTAGMUS	REMARKS
178	7/15/66	S:166.7	167.2	167.3	169.7	170.3	166.6	144	138	R-H
		D:119.8	118.5	121.4	126.4	124.0	118.6			
"Anesthetized"										
		S:169.7	162.7	162.8	165.9	164.3	161.0	174	168	R-C
		D:122.7	111.9	118.8	122.4	118.5	115.6			
		S:160.7	157.2	162.5	164.0	165.7	167.0	156	168	L-C
		D:114.3	110.2	116.5	117.8	120.8	121.7			
		S:168.4	167.4	168.2	169.1	172.7	172.3	153	164	R-C
		D:121.6	120.1	122.4	123.0	126.5	124.6			
		S:173.2	169.3	167.1	172.8	170.4	166.6	195	183	L-C
		D:125.3	118.3	120.8	126.7	124.0	120.4			
"	7/19	S:145.2	154.0	145.7	147.1	147.1	145.8	225	212	R-C
Awake		D: 81.1	81.2	83.7	85.2	82.4	77.0			

TABLE 3

MONKEY	DATE	A IRRIGATION BEFORE	A IRRIGATION AFTER	NYSTAGMUS BEGINS BEFORE	NYSTAGMUS BEGINS AFTER	SEVERE NYSTAGMUS DURING	NYSTAGMUS ENDS	B HEART RATE CONTROL	B HEART RATE NYSTAGMUS	REMARKS
181	7/18/66	S:159.1	148.9	156.3	159.9	153.6	160.6	-	-	R-C
		D:109.6	102.5	109.4	112.9	106.9	110.6			
"Anesthetized"										
		S:150.6	148.9	160.4	160.3	146.1	146.1	-	-	L-H
		D:106.0	103.0	113.8	113.3	103.9	104.0			
"	7/20	S:162.1	169.9	199.4	199.4	191.1	166.0	-	-	R-H
		D: 93.7	94.0	106.8	110.8	104.0	95.9			
Awake										
		S:188.5	180.0	201.4	201.7	205.6	203.6	-	-	R-H
		D:105.3	96.2	114.4	114.4	116.7	112.9			
		S:172.5	168.0	185.1	192.9	201.6	188.8	-	-	L-H
		D:102.9	101.9	94.0	105.3	116.1	106.7			
		S:180.4	177.2	199.7	202.3	212.8	199.1	-	-	R-H
		D:117.2	116.3	120.0	121.8	131.4	125.6			
		S:197.3	All	202.9	202.8	196.5	181.2	-	-	L-H
		D:120.6	Artifact	123.8	123.7	124.3	121.2			
		S:165.9	173.4	184.5	185.2	186.6	179.6	-	-	R-C
		D:111.7	113.2	119.3	118.2	118.6	112.4			
		S:198.8	214.4	202.0	202.7	200.1	191.2	-	-	R-C
		D:115.3	128.3	116.0	115.8	117.0	110.6			
		S:179.7	All	187.6	190.2	177.0	179.1	-	-	R-C
		D:112.3	Artifact	110.5	112.5	110.8	112.9			

RELATIONSHIP BETWEEN RESPIRATORY RATE, TIDAL VOLUME AND CARDIAC CONDITIONING (In Progress).

Studies are now in progress to develop adequate means of recording respiration in unanesthetized primates and dogs. Previously we have used a circumthoracic strain gage belt to measure respiratory rate. This method of recording respiratory rate has been very useful, but it is sensitive to movement artifacts.

Recently we have developed techniques for recording respiration with thermocouples which have been implanted in the trachea. The advantages of using implanted thermocouples are: 1) they can determine rate and to some extent volume during respiration; 2) they can be used for EKG by recording from one side of the thermocouple wire to a ground wire; 3) body temperature can be obtained with calibrated thermocouples; and 4) they are less sensitive to movement artifact because they are fixed to the trachea.

Figure 1A illustrates a tracing obtained with a thermocouple pick-up which have been in the trachea for 14 days. Note that an EKG is recorded from thermocouple to ground lead. Eigure 1B illustrates the surgical steps for implanting a thermocouple pick-up in the trachea.

Fig. 1A

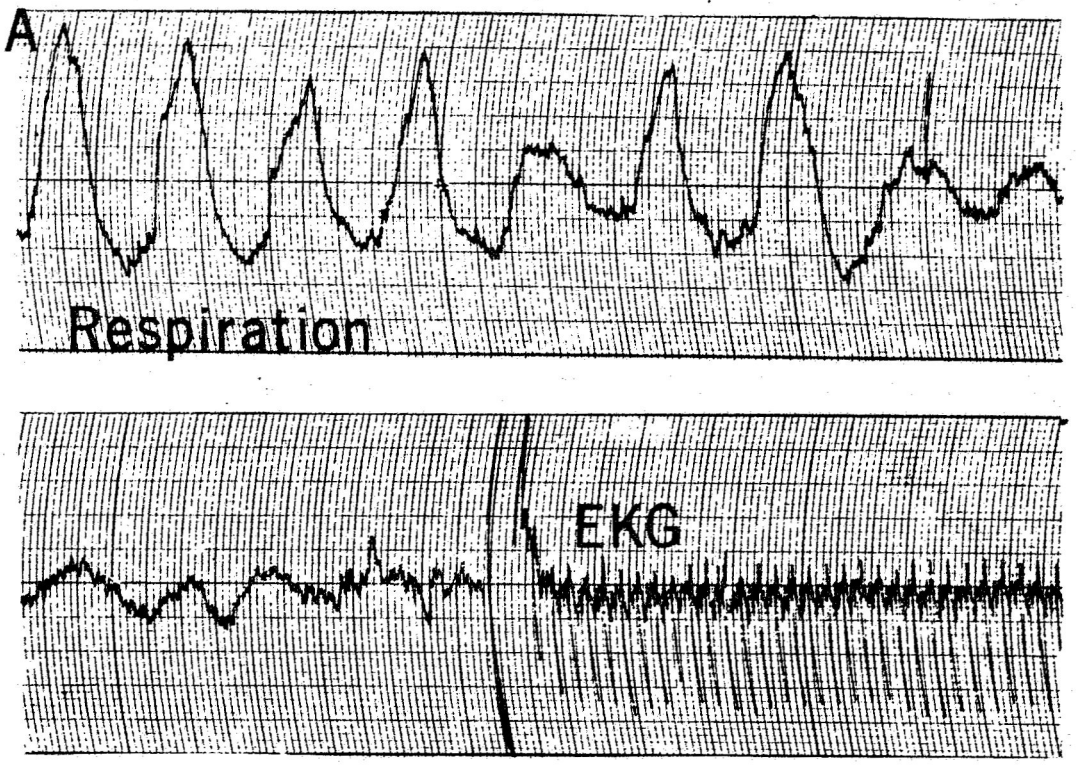
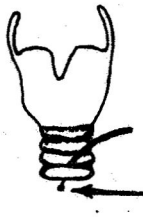


Fig. 1B



Thermocouple Pick-Up

STUDIES OF SINUS ARRHYTHMIA

The study of the relationship of heart rate to respiration is pertinent to investigations concerning cardiac conditioning. There have been, a number of investigations showing that respiration plays an important role in human cardiac conditioning as shown by Westcott and Huttenlocher (1961) and Woods and Obrist (1964). Other investigations have shown that respiration can also be employed as an unconditional stimulus to establish cardiac conditional reflexes in humans (Perez-Cruet, 1962).

In studies by Perez-Cruet and Gantt (1961) where the relation between respiration and heart rate was investigated in normal and healthy dogs they found that sinus arrhythmia persisted even when there were marked acclerations of respiration due to panting or after drugs which produced muscular inhibition and cardiovascular changes (Perez-Cruet and Gantt, 1959, 1964). These investigators also found that the fluctuations of heart rate which follow a given respiratory pattern can continue in many instances even when the respiratory rate changes rapidly. They concluded that sinus arrhythmia can follow a rhythmical heart rate fluctuation parallel to a former pattern of respiratory rate and that it can occasionally be unaltered during rapid changes of this rate. In other words, in some dogs, where the pattern of sinus arrhythmia follows precisely the slow respiratory rate, the sinus arrhythmia pattern persists unchanged when the respiration changes to a rapid panting. Therefore, respiratory rate determines fluctuations of heart rate under usual conditions and this usual pattern in impressed on the heart rate in preference to a fluctuation based on a sudden alteration of respiratory rate. These original observations on the presence of sinus arrhythmia during extreme forms of breathing such as panting in dogs were later elaborated by recent studies (Perez-Cruet, Newton and Gantt, 1965).

Figure 1 illustrates an example where the pattern of sinus arrhythmia, previously associated with a regular slow breathing, continues during rapid breathing (panting) at 110 respiratory cycles per minute (marked - short panting in the illustration) and even during a more persistent episode of rapid breathing (marked prolonged panting) at respiratory rates of 150 and 160 cycles per minute. Note that during the prolonged panting there is a tendency for the sinus arrhythmia to diminish but that the previous respiratory pattern imposed on the heart rate fluctuation still persists.

Figure 2 shows sinus arrhythmia in another dog during panting in which the fast respiratory movements are deep or shallow which in this particular tracing illustrates no clear relationship between depth of breathing during panting and sinus arrhythmia.

In our studies in unanesthetized dogs isolated in sound proof rooms, sinus arrhythmia is a very common phenomenon. It is our impression that the presence of sinus arrhythmia in these animals indicates that the animals are not ill or frightened by the procedures. This impression is supported by studies that have shown that sinus arrhythmia is very common in healthy undisturbed dogs (Detweiler, 1960).

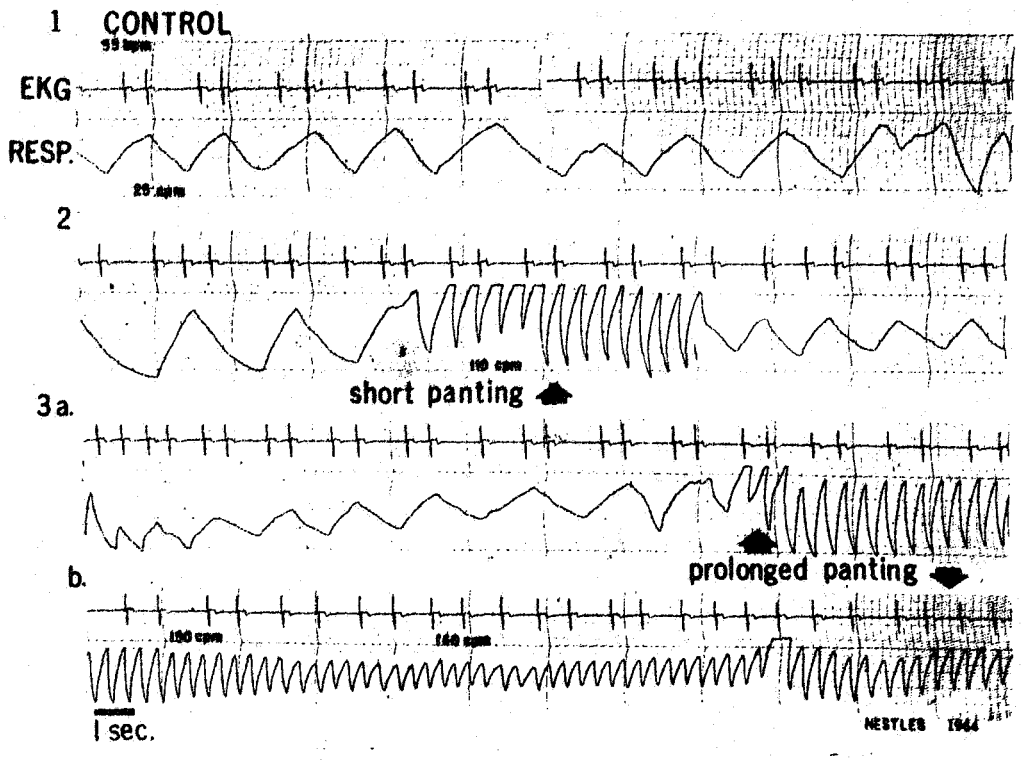


Figure 1. Sinus arrhythmia during short and prolonged panting in an unanesthetized dog isolated in a soundproof room. Tracing 1, at the top, is a control record of EKG (chest lead) and respiration (Resp.) . Respiration was measured with a circumthoracic strain-gauge respirometer. Control heart rate is 55 beats per minute and control respiration is 25 respiratory cycles per minute. Tracing 2, middle tracing, illustrates sudden appearance of panting for 7 seconds. Note that a two-beat sinus arrhythmia previously seen during the control period and prior to panting still persists even when respiration has changed abruptly into a rapid respiratory rate. Tracings 3 a and b illustrates the presence of sinus arrhythmia during more prolonged panting for 31 seconds. Note that sinus arrhythmia is still evident in spite of the increase in respiratory rate.

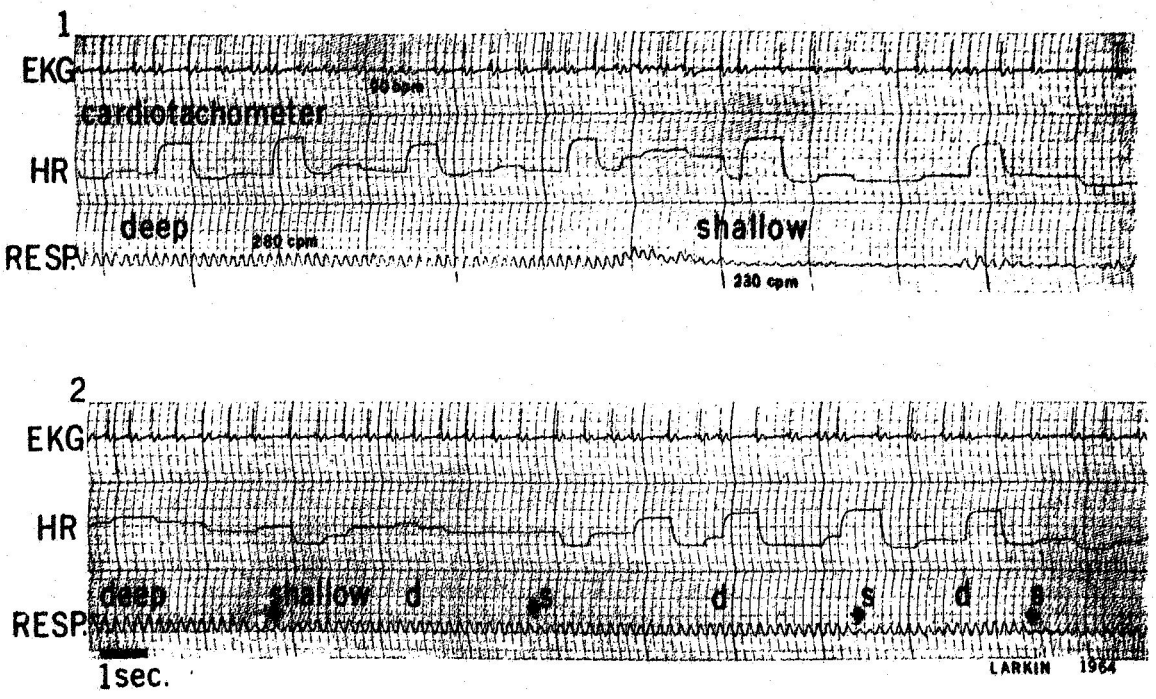


Figure 2. Sinus arrhythmia during deep (D) and shallow (S) panting. Tracing 1 illustrates EKG (chest lead); heart rate, beat-to-beat from a Gilford cardiotachometer; and respiration (Resp). Amplitude of the respiratory curve represents depth of breathing during panting. Respiration was measured with a circumthoracic respirometer. Respiratory rates during panting varied from 230 to 280 respiratory cycles per minute. Tracing 2 shows same physiological measurements as in tracing 1 except that deep and shallow panting occur in succession more frequently than in the first tracing. Note that sinus arrhythmia still is present during deep or shallow panting at rates of 230 to 280 respiratory excursions per minute.

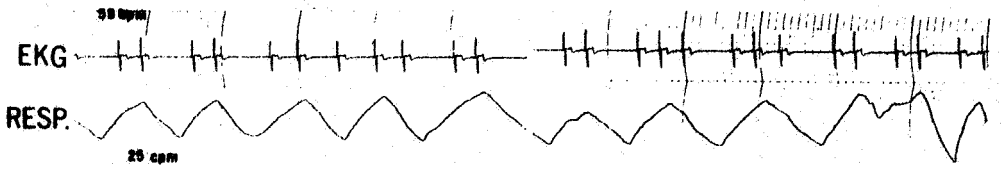


Figure 3. Two-beat sinus arrhythmia in phase with respiration. Note that after a long pause two beats with normal p waves (sinus beats) occur in succession followed by another long pause.

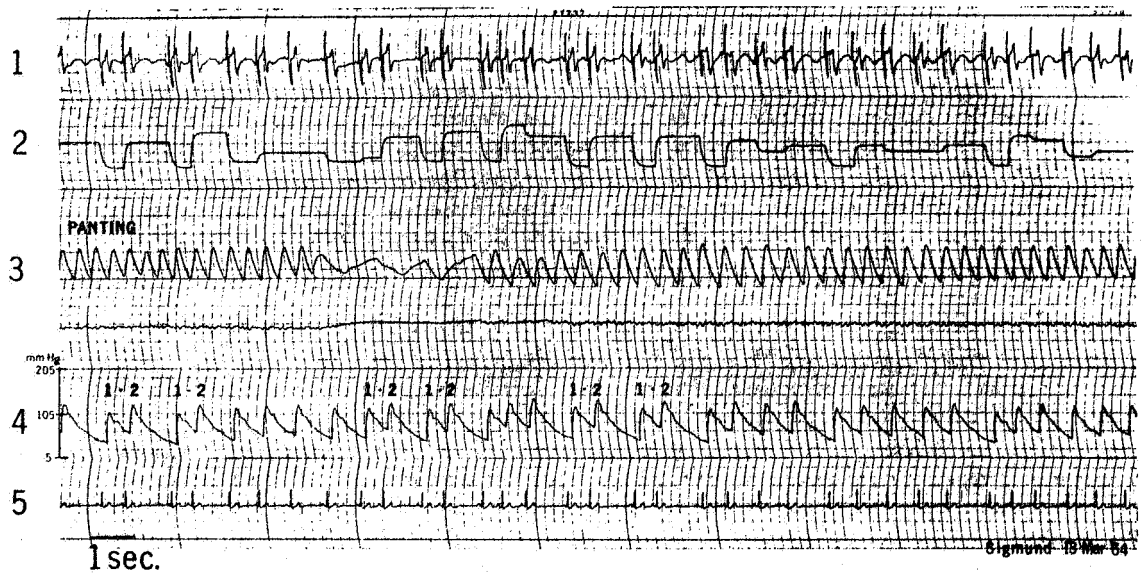


Figure 4. Dissociation of sinus arrhythmia and respiration. The tracings also illustrate two-beat sinus arrhythmia during panting. 1. EKG (chest lead); 2. heart rate, beat-to-beat from a Gilford cardiotachometer; 3. Respiration (tracing immediately below the respiration channel is a movement indicator channel). 4. Direct intra-arterial blood pressure in mm. Hg.; and 5. standard EKG (lead II). Note that during the two-beat sinus arrhythmia there is an increase in systolic blood pressure during the fast beat (marked with a 2 on top of the blood pressure tracing).

The most typical sinus arrhythmia consists of progressive acceleration in heart rate followed by deceleration which usually, but not always, is in phase with respiration. The sinus arrhythmia usually occurs in multiple patterns and the number of heart beats within a respiratory cycle can vary between three heart beats or two R to R intervals to as many as 12 or more R to R intervals within a long respiratory cycle. The number of R to R intervals within a given sinus arrhythmia cycle is called a two-beat sinus arrhythmia described originally by Perez-Cruet, Newton and Gantt (1964). Two-beat sinus arrhythmia is a special type of arrhythmia in which after a long pause, the sinoauricular node initiates two successive beats followed by another pause. This type of sinus arrhythmia resembles cases of premature contractions, such as coupling, but it differs from the last in that the beats are of sinus origin. Figure 3 illustrates an example of two-beat sinus arrhythmia in phase with respiration. We have observed this type of two-beat sinus arrhythmia in about 80% of our dogs. It usually occurs sporadically and in many instances is associated with a fast respiratory rate. This type of sinus arrhythmia is usually accompanied by changes in blood pressure as shown in Figure 4. Note that the systolic blood pressure increases during the second heart beat (fast beat) in about 80% of the dogs shown in this figure, but in some cases the systolic blood pressure decreases. We do not have any definite explanation as to why the systolic blood pressure usually increases and at other times decreases. At first it was postulated that these changes in blood pressure were entirely associated with a critical heart rate interval, that is, very fast heart rates interfered with cardiac filling causing a decrease in systolic pressure. However, even under these conditions on many occasions we observed an increase in systolic blood pressure with the fast beat. We also found that the increase in systolic blood pressure during the fast beat occurred more often in recordings from the central ascending aorta than from the peripheral or abdominal aorta, when these pressures were recorded simultaneously through individual intra-aortic catheters in the same animal. Figure 5 illustrates a number of blood pressure determinations from the ascending and abdominal aorta in two dogs, Hobo and Jeff. Note that in Hobo the central systolic pressure increases with the fast beat whereas the peripheral systolic pressure shows less instances with such an increase during the fast beat. Similarly in the dog Jeff, there is a definite tendency for the systolic pressure to increase during the fast beat from recording of central pressures but this tendency is much less in the recordings from the abdominal aorta. Another finding is that while the systolic pressure increases or decreases during the fast beat in two-beat sinus arrhythmia, there is a definite <u>decrease</u> in aortic and carotid blood flows, as well as in the amplitude of the optical plethysmogram, as shown in Figure 6 and 7.

Although the mechanisms for the increase in systolic pressure during two-beat sinus arrhythmia are not known there has been interpretations made by Woodbury on similar blood pressure changes seen with premature auricular contractions in children during which the systolic pressure increased during the fast beat probably due to an increase in atrial filling.

During sinus arrhythmia with more prolonged cycles the differences between the average heart rate at the low point (first beat of the cycle) and the peak of the cycle can range from 19 to 94 beats per minute. The differences between the average systolic and average diastolic blood pressure of the first beat and the peak of the cycle can range from 2 to 38 mm Hg. How are we then to separate these significant increments in heart rate and

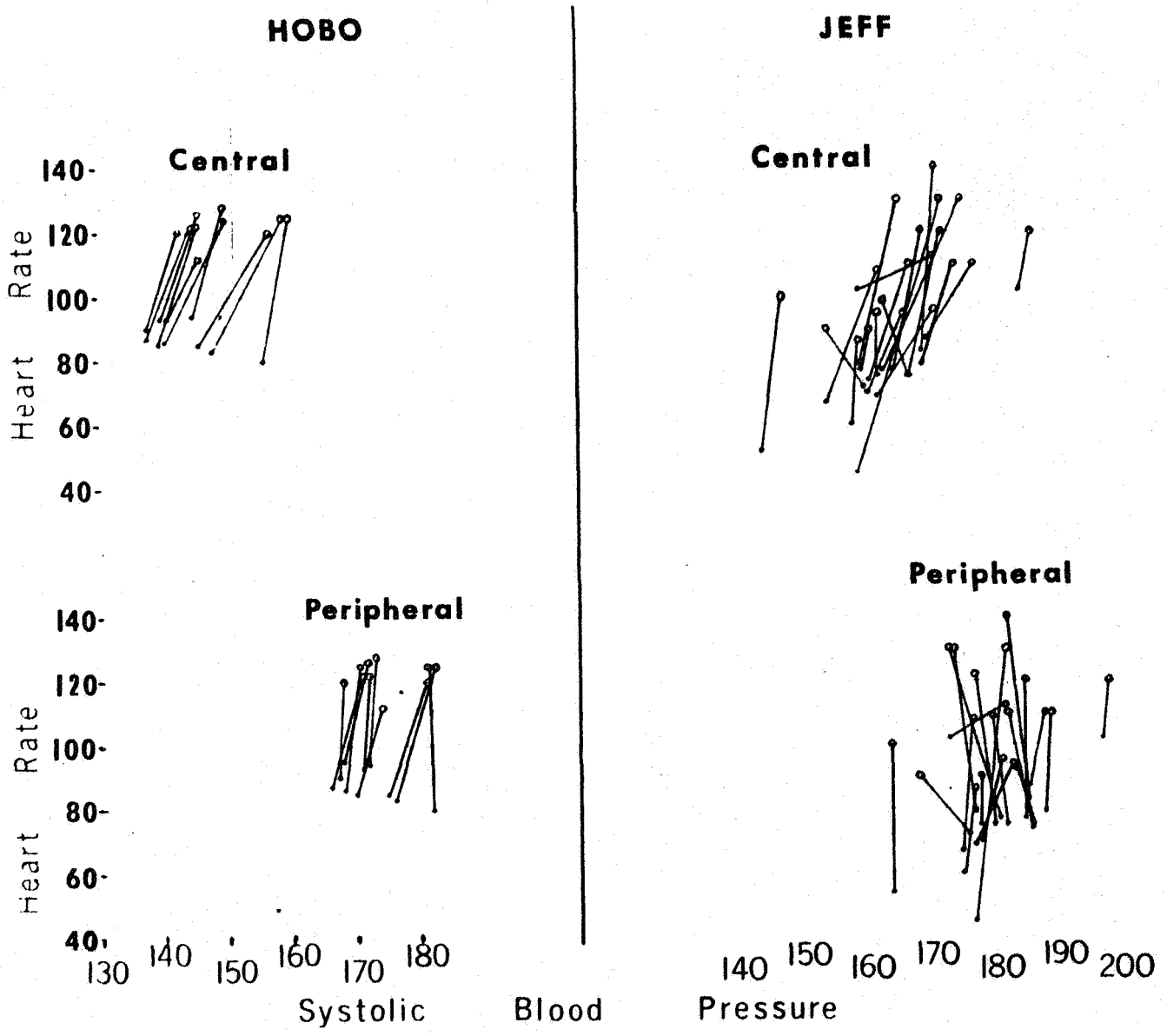


Figure 5. Two-beat sinus arrhythmia: comparison of central and peripheral blood pressures on individual beats in two dogs (Hobo and Jeff). Ordinate represent the heart rate (R-R interval) in beats per minute; abscissa represent direct intra-arterial blood pressure. Central and peripheral blood pressure from ascending and abdominal aorta respectively. Close dot(•) represent first (slow) heart beat and open dot(○) second (fast) heart beat during two-beat sinus arrhythmia. Note that the systolic aortic pressure increases more consistently during the fast beat in the pressure readings from the ascending aorta.

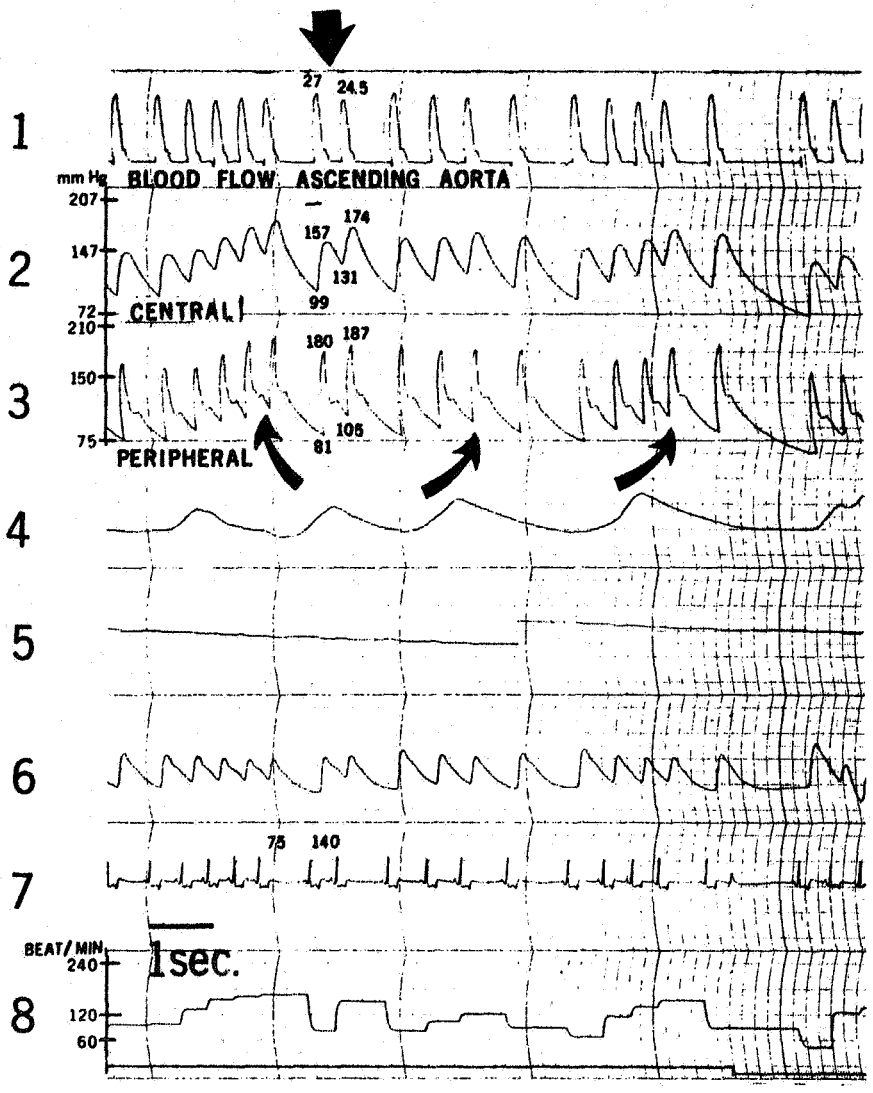


Figure 6. Cardiovascular changes during sinus arrhythmia. 1. Blood flow from ascending aorta using an electromagnetic flow probe which has been implanted for one month; 2. Central (intra-arterial) blood pressure from ascending aorta; 3. Peripheral (intra-arterial) blood pressure from abdominal aorta; 4. Respiration; 5. Flow integration curve; 6. Impedance plethysmogram; 7. EKG (lead II); and 8. Heart rate, beat-to-beat from Gilford cardiotachometer. Note that during a two-beat sinus arrhythmia (marked with black arrow) the pulsatile aortic blood flow amplitude is reduced slightly, but heart rate, central and peripheral systolic pressures are increased during the second beat. Note also that the maximum level of diastolic pressure and heart rate at the peak of the sinus arrhythmia cycle preced the maximum systolic pressure during longer cycles of sinus arrhythmia as shown with curved arrows.

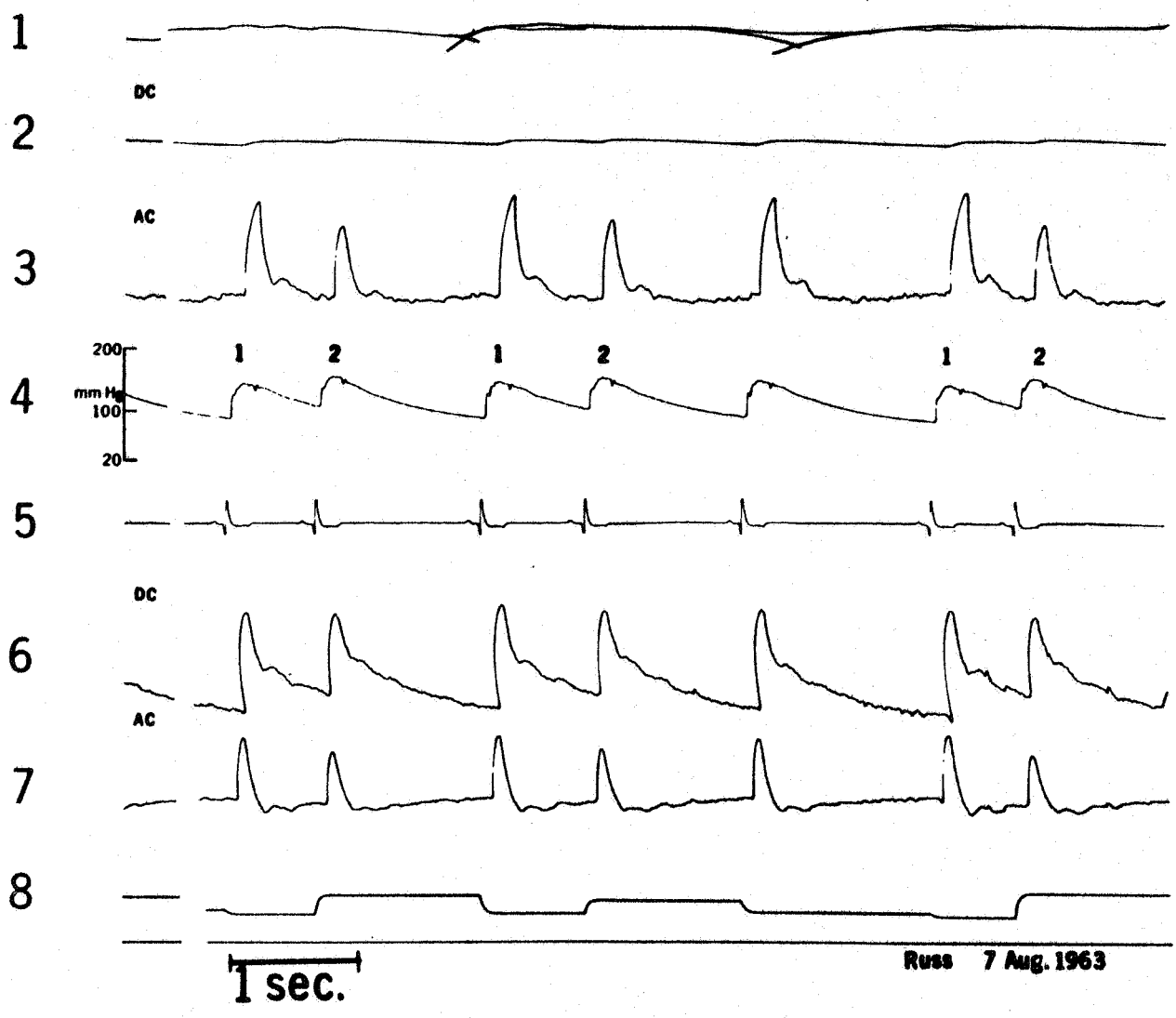


Figure 7: Changes in carotid blood flow (electromagnetic) and central blood pressure during two-beat sinus arrhythmia. 1. Respiration 2. DC optical plethysmogram 3. AC optical plethysmogram 4. central blood pressure from the ascending aorta 5. EKG (lead II) 6. Blood flow through internal carotid artery recorded with a DC coupler 7. Blood flow (internal carotid) recorded with an AC coupler. Time constant set a 0.03 second. Note that during two-beat sinus arrhythmia cycles, marked 1 and 2 on the blood pressure tracing, the systolic pressure increases slightly, but the pulsatile carotid blood flow and optical plethysmogram decrease.

blood pressure from those observed during cardiac conditioning? In our studies we have found that sinus arrhythmia follows a given pattern which is usually, but not always, associated with respiration. During conditioning, however, the cardiac changes are more exaggerated than those observed at the peak of the sinus arrhythmia cycle. Also by averaging techniques, described in previous reports, cancellation of the irregularities due to sinus arrhythmia is accomplished after many sinus arrhythmia cycles have been added and averaged. However, the sinus arrhythmia contributes definitely to high standard deviations and wide range of values in histograms of cardiovascular functions such as heart rate and blood pressure.

Although the exact mechanisms of sinus arrhythmia are not known, we have found a number of relationships which suggest that sinus arrhythmia can be influenced by factors other than respiration. For example, we have not only observed sinus arrhythmia during panting, as shown in Figures 1 and 2, but we have also seen it during breath holding during diving under water in iguanas (green iguanas). Vallbona, et al. (1965) in a separate study have also observed sinus arrhythmia during apnea and they also observed sinus arrhythmia at the same periodicity as before the apnea. Also hemodynamic factors which probably stimulate receptors within the heart are involved in the genesis of sinus arrhythmia, because it has been shown that the maximum heart rate and diastolic pressure levels always preceded the maximum systolic pressure at the peak of the sinus arrhythmia cycle (Perez-Cruet, 1965).

One of the most interesting findings of studies which are now in progress, is the appearance of sinus arrhythmia in dogs with complete mid-cervical vagotomies which have been treated with propranolol (Inderal). The central mediation of sinus arrhythmia has been attributed entirely to the vagus nerve and these preliminary observations in a vagotomized animal may suggest that even in the absence of the vagus nerve and blockage of the beta adrenergic receptors in the heart, one can still observe sinus arrhythmia. How are we to explain sinus arrhythmia in the vagotomized dog where the vagus nerve has been completely resected in the neck? It appears from our preliminary observations that sinus arrhythmia is a rate-dependent phenomenom, that is, if the heart rate is too fast then the sinus arrhythmia diminishes. Slowing of the resting heart rate usually permits sinus arrhythmia to reappear. In the vagotomized dogs, where the heart rate can vary very little but where rates vary from 135 bpm in some dogs to 250 bpm in others, fluctuations in heart rate associated with respiration have been observed only in those dogs with low resting heart rate levels.

From the above observations it appears that there are intrinsic mechanisms within the heart which can produce sinus arrhythmia. These intrinsic mechanisms are probably similar to those described elsewhere by James (1963) who has shown that an increase in perfussion pressure through the sinoauricular artery produces a bradycardia due to stimulation of receptors within the sheath of the artery supplying the sinoauricular node. It is possible to postulate that the increment in diastolic pressure during the peak of the sinus arrhythmia cycle is one possible triggering mechanism for initiating the deceleration of heart rate during sinus arrhythmia.

More recently, in some dogs paralyzed by d-tubocurarine or succinylcholine, fluctuations in heart rate can be in or out of phase with artificial respiration where the depth and rate are kept constant. These phase relationships with respiration can shift during a given experimental session so that an independence or a dependence can be established (studies of Newton and Perez-Cruet).

Instances of dissociation of sinus arrhythmia from respiration in dogs under curare or succinycholine suggest that chest expansion is not the critical factor in the genesis of the sinus arrhythmia. According to Clynes' (1960) studies, pulmonary stretch receptors should be responsible for sinus arrhythmia. However, on account of the numerous experiments already presented in this session, it is clear that this is not the only mechanism.

BLOOD PRESSURE AND HEART RATE CHANGES DURING SINUS ARRHYTHMIA.

The most typical sinus arrhythmia (SA) consists of a progressive acceleration in heart rate (HR) followed by deceleration which is usually, but not always, in phase with respiration. SA cycles always have two or more sinus beats. Two-beat SA is commonly seen in dogs (Perez-Cruet, et al, Physiologist, 7: 222, 1964). In this study, the analysis of SA cycles of 4 or more beats was done in 7 dogs. Fifty SA cycles were selected at random from each dog. Records had been taken with the dog standing quiet in a soundproof room at 74° F. HR was measured with a R-R ruler in bpm. Direct BP from the lower abdominal aorta was measured with a Statham transducer. Four dogs showed a higher incidence of 5 beat SA cycles (38 to 60%) and 6 beat SA cycles (12 to 36%), while the rest showed a higher incidence of 7, 9 and 11 beat SA cycles (22, 34 and 26%). The HR and BP increased during the accelerative phase of SA and decreased during the decelerative phase. Phasic lags were found between the HR and BP curves. The differences between the mean HR of the first beat and the peak of the cycle ranged from 19 to 94 bpm. The differences between the mean systolic and mean diastolic BP of the first beat and the peak of the cycle always preceded the systolic BP peak. The study of SA using the number of beats in SA cycles may prove useful in elucidating some mechanisms concerning the genesis of this arrhythmia.

SINUS ARRHYTHMIA DURING PANTING.

We have previously reported that sinus arrhythmia can be independent of respiratory rate under the action of drugs and emotional stress (Fed. Proc. 20: 89, 1961). In 8 unanesthetized dogs isolated in a soundproof room, the relation between sinus arrhythmia and panting was investigated. These dogs have been trained for many weeks to stand quietly in a soundproof room. It was found that the rhythmical changes in heart rate which normally accompanies the respiratory cycle (sinus arrhythmia) was usually but not always diminished or abolished during rapid panting. The present studies confirm the fact that sinus arrhythmia can continue in many instances when the respiratory rate changes suddenly to panting. On many occasions where sinus arrhythmia persisted during panting the pattern of rhythmical heart rate fluctuations was parallel to the pattern seen during the preceding respiratory rate. The present work shows that sinus arrhythmia can be independent of respiratory rate when respiration changes to rapid panting. Under certain circumstances the pattern can be determined by factors other than respiration.

This study is a continuation of previous investigations designed to elucidate the effect of emotional stress on normal rhythmic functions of the cardiovascular system in dogs.

SYSTOLIC BLOOD PRESSURE CHANGES DURING TWO BEAT SINUS ARRHYTHMIA.

Two beat sinus arrhythmia is a special type of arrhythmia in which, after a long pause, the sinoauricular node initiates two successive beats, followed by another long pause. This type of arrhythmia resembles cases of premature contractions (coupling). We have observed this type of arrhythmia in about 80% of our dogs. It usually occurs sporadically and in many instances is associated with a fast respiratory rate. In seven unanesthetized dogs, direct systolic blood pressures were measured during two beat sinus arrhythmia. During the second beat the systolic pressure was increased 4 to 10 mm Hg from the pressure level of the first beat except in one dog in which the systolic pressure always decreased during the second beat. The incidence of systolic blood pressure increase on the second beat is greater in central pressures (90 to 100% of occurrences) than in peripheral pressures (43 to 80%). Three mechanisms are responsible for the increase in blood pressure during the second beat: 1) changes in cardiac output, 2) peripheral resistance, and 3) cardiac rate. Respiratory rate is not necessarily responsible for these changes (Perez-Cruet, et al., Fed. Prof. 20, 1961, p. 89). During the second beat the stroke volume is usually decreased as well as the amplitude of peripheral optical plethysmography. The mechanism for the consistent decrease in systolic pressure in the second beat during two beat sinus arrhythmia in one dog is not known and is under investigation. In summary, the study showed that usually in two beat sinus arrhythmia the systolic blood pressure increases slightly during the second beat.

RESPIRATORY SINUS ARRHYTHMIA IN DOGS WITH CHRONIC BILATERAL CERVICAL VAGOTOMIES.

Sinus arrhythmia is usually, but not always, influenced by respiration. Sinus arrhythmia in dogs has been shown to be independent of respiration during severe panting as reported elsewhere by Perez-Cruet (1961) and also during apnea as observed by $V_a$llbona, et al. (1965). Vagal influences are undoubtedly of importance in the mediation of respiratory sinus arrhythmia in intact dogs. In a study designed to investigate cardiovascular conditioning in vagotomized dogs, we made a series of observations of changes in heart rate accompanying respiration similar to those observed during respiratory sinus arrhythmia. A total of 7 dogs in whom the vagosympathetic nerve has been bilaterally cut at the level of the third cervical vertebra were studied. In 4 dogs there was definite evidence of sinus arrhythmia synchronous with respiration. All dogs that showed sinus arrhythmia developed the accelerative phase of sinus arrhythmia during expiration whereas in normal dogs the accelerative phase of sinus arrhythmia usually occurs during inspiration. The changes in heart rate from the trough to the crest of the accelerative phase varied between 10 to 20 beats above the lowest heart rate level.

This study shows that sinus arrhythmia can occur even after the vagi nerves are cut and that under these conditions the accelerative phase paradoxically occurs during expiration rather than during inspiration indicating a shift in the cycling of sinus arrhythmia.

HEART-RATE AND BLOOD-FLOW CHANGES ACCOMPANYING ATTEMPTED RENAL CONDITIONING IN DOGS.

Previously it has been shown in this laboratory that conditional diuresis could not be conditioned in dogs with denervated kidneys homotransplanted into the neck (Baker, et al., Fed. Proc. 1964). The purpose of this study was to determine if a conditional water diuretic response could be established in dogs with innervated kidneys and also to investigate other physiological parameters, such as renal blood flow (electromagnetic) (RBF) and heart rate (HR) accompanying renal conditioning. A total of 15 mongrel dogs were used in these studies. All animals were deprived of water for at least 24 hours prior to the experiments. Techniques for collecting urine directly from the ureter were used in most dogs as described elsewhere (Perez-Cruet and Gantt, Nephrology Congress, p. 254, 1966). Two experimental procedures were used. In the first procedure, tone of 6 sec. duration were presented alternately at intervals of 2 to 3 min., 10 to 20 times during a session. A tone 512 cy/sec. was always reinforced with 5 to 20 cc. of water. Another tone, 256 cy/sec. was never reinforced. In the second procedure, the animals were adapted daily to an experimental room for 5 to 15 minutes at which time a tone 256 cy/sec. was presented for 1 min. followed 5 min. later by another 1-min. tone, 512 cy/sec. and reinforced with 1000 cc. of water. The animals were allowed to drink ad lib. within a 15-minute period. In these experiments the subjects were retained in the room from 1 to 3 hours. Results showed that with procedure number one, unstable renal conditional diuretic responses were obtained. There were definite conditional increases in conditional RBF and HR. Differentiation was observed in these cardiovascular measures. No renal conditioning was obtained with procedure number two. In both procedures drinking water produced two types of unconditional diuretic responses. In procedure one, there was an immediate, but transient, diuresis; with procedure two, there was a water loading diuresis which appeared 15 to 45 minutes after drinking water ad lib. The mechanisms for the unconditional diuretic response with procedure one, probably depends on an increase in glomeruler filtration rate or due to mechanical activation of ureteral contraction. In procedure two, the unconditional diuresis was equivalent to the well known loading water diuresis. In conclusion, the study demonstrates definite conditional changes in RBF and HR with water as the unconditional stimulus, but renal conditional diuresis was not clearly established. It is possible that the dehydration in these experiments was a possible factor in the inability to form renal conditional diuresis.

THE EFFECT OF NOVEL STIMULI ON RENAL SECRETION COLLECTED BY THE URETERAL-SHUNT METHOD.

Experiments designed for the study of renal secretion have usually involved collecting the urine from externalized or acutely catheterized ureters or bladder fistulas. The purpose of this study is to present the results of a method for collecting urine using a ureteral-shunt, and to investigate changes in renal secretion to novel stimuli (new environmental conditions or auditory signals). Six unanesthetized dogs, isolated in a soundproof room, were used. Urinary secretion was measured in drops per 6 sec. intervals. There was marked variability in the secretion of urine from the right and left kidneys. One dog showed marked changes in renal secretion which were associated with a new environment. Four of the dogs showed slight increases in urinary secretion during or after auditory signals (T256, T512) which had never been reinforced with liquids. Great variability in these changes were observed from day to day, but a trend to extinction by repetition was evident in some dogs. The data suggest that the changes in renal secretion might be similar to other autonomic components usually associated with a response to novel stimuli, viz. the orienting reflex.

THE EFFECT OF CARDIAC ARREST WITH CLOSED CHEST MASSAGE ON CLASSICAL CONDITIONAL REFLEXES.

Some patients after a cardiac arrest and external cardiac massage have developed transient episodes of memory loss (unpublished observations). This study was designed to determine if prolonged periods of 10 to 15 minutes of cardiac arrest with closed chest massage have an effect on the ability to retain classical cardiac and motor conditional reflexes.

Since Gantt has shown that the cardiac conditional reflex is a reliable index of the integrity of cortical functions (Autonomic responses in differential diagnosis of organic and psychogenic psychoses. Arch. Neurol. & Psychiat., 70, 778, 1953) the study was designed to determine, using cardiac conditional reflexes as an index, if some evidence of memory loss or cortical functions have been affected.

A total of 8 dogs have been used in this study. Three dogs died during the final arrest procedure and four dogs have survived 10 and 15 minutes of cardiac arrest. The dogs had well established motor and cardiac conditional reflexes by the time they were exposed to the cardiac arrest and closed chest massage procedure.

All dogs showed some impairment of motor and cardiac conditional reflexes 24 to 48 hours after 15 minutes of cardiac arrest and closed chest massage, but this capacity was definitely regained after 72 hours. In one dog, the effect of anesthesia, electric shock through the heart, and operative procedures, were ruled out as being the main factor in this temporary loss.

THE EFFECT OF CARDIAC ARREST WITH CLOSED CHEST MASSAGE ON THE EEG IN DOGS.

We have previously shown that prolonged cardiac arrest with closed chest massage (CCCM) results in a temporary loss of conditional reflexes in dogs (Clin. Res., XII: 440, 1964). In the present study, control EEGs from parietal, frontal and occipital cortex were taken in seven dogs anesthetized with pentobarbital. Cardiac arrest was then produced by electric shock. CCCM was begun after the EEG markedly decreased in amplitude during the early stages of fibrillation. After variable periods of CCCM, short runs of EEGs were taken. After 15 minutes an electric countershock was used to defibrillate the hearts, and additional EEGs were taken. Low-voltage (4 to 20 μv), low-frequency (5 to 13 per second) waves were observed after cardiac arrest. During the first 30 seconds of fibrillation, spikes were observed and the amplitude of the EEGs usually was reduced to about 2 μv. In four dogs, an almost flat EEG was temporarily restored to a low-voltage, low-amplitude EEG, similar to that observed during the control periods, after periods of 1½ or more minutes of CCCM. The EEGs taken after defibrillation showed no significant deviation from the control EEGs with respect to either frequency or amplitude. These observations suggest that the diminished electrical activity present in the EEG of the clinically dead dog can be restored temporarily to control levels by closed chest massage.

INABILITY TO FORM CARDIAC CONDITIONAL REFLEXES IN THE "DENERVATED" HEART.

Previous work by Dykman and Gantt (J. Comp. Physiol. Psychol., 52: 163, 1959) has shown that moderate doses of atropine did not abolish the cardiac conditional reflexes, however, atropine produced an elevated heart rate (HR) and restlessness. The present study was designed to investigate if surgical chronic cervical vagotomy plus beta adrenergic blockade with propranolol in doses of 1 to 5 mg/kg, could be used as a model for pharmaco-surgical "denervation" of the heart. Five dogs were used in this study. Cardiac conditional reflexes were well established in the vagotomized dogs as reported elsewhere (Perez-Cruet and Gaertner, Fed. Proc., 26: 328, 1967). Propranolol in doses of 1 mg/kg produced base-line HR levels comparable to the denervated heart (70 to 100 beats per min.) After "denervation" with propranolol HR conditional reflexes (HR-CRs) were completely abolished. These results showed that peripheral adrenergic mechanisms mediating the HR-CRs were completely blocked in the "denervated" heart. The study suggest that neurohumoral agents other than those at the beta receptor site were not primarily mediating the HR-CRs in the vagotomized dogs. The study also indicates that the beta receptor mechanisms play an important role in the mediation of HR-CRs in vagotomized dogs.

INABILITY TO FORM CARDIAC CONDITIONING IN THE DENERVATED HEART.

Neurohumoral mechanisms have been implicated in the genesis of cardiac conditioning (Bykov, 1957). The mediation of the cardiac conditional reflex is a complex one probably involving neurohumoral elements within the heart. There also may be a possibility that neurohumoral secretions by the adrenal or other storage organs might influence the cardiac conditional reflex.

The present study was designed to determine if cardiac conditioning could be formed in a heart which had been denervated surgically and pharmacologically.

Four dogs have been used. All animals had established cardiac conditional reflexes prior to the denervation.

The four dogs with cardiac denervation have not shown any evidence of cardiac conditioning. These preliminary results indicate that the cardiac conditional reflex is mediated by the sympathetic or parasympathetic system and is not entirely dependent on adrenal or other neurohumoral factors. The study does not rule out, however, that in the normally innervated heart neurohumoral factors play an important role in the magnitude and direction of the cardiac conditional reflex.

NORMAL ELECTROCARDIOGRAMS IN DOGS.

Numerous reports have concluded that there is no normal electrocardiograms in dogs. Preliminary studies in this laboratory confirmed these results when standard clinical methods of electrocardiography were applied. It was found that a recovery RC time constant of 1 second, as used in most electrocardiographs, is not suitable for research canine electrocardiography in our experiments. The two main problems in recording ECGs using this time constant are: one) a wandering baseline subject to severe drifts when the animal moves, and two) accentuation of variations in waveform which are normally accentuated in the dog.

Canine electrocardiograms taken at various time constants: 1, 0.3, 0.1 and 0.03 second showed that the last time constant of 0.03 sec. was more suitable for these experiments. Time constants of 1 second are very useful in detecting variations in the amplitude of the P and T waves as well as detecting changes in depressed ST segments. Settings at a time constant of 0.03 sec. do not show T wave changes as well as setting of 1 second time constant.

Either 1 or 0.03 second time constant are useful in the study of PR intervals and durations.

Research canine electrocardiography should include at least one lead (preferably limb lead 2) at a time constant of 1 second and additional standard limb and chest leads at a time constant of 0.03 second.

Recordings have been carried out in several dogs with recordings of electrocardiograms at a time constant of 0.03 second. Recordings in these animals have shown less variations in the ECG waveform.

EFFECT OF PERSON ENTERING THE EXPERIMENTAL ROOM IN DOGS WITH AV BLOCK.

In five dogs, we have determined the effect of a person entering the room on cardiac activity in dogs with AV block. The results show that when a person enters the room, there are marked changes in both auricular and ventricular rates. Studies are in progress to determine the mechanisms of this response.

EFFECTS OF EXTERNAL CATHODAL POLARIZATION ON CLASSICAL MOTOR CONDITIONING IN DOGS.

In normal or depressed subjects an external polarizing electric current passed through the brain appears to cause euphoria if the positive polarity is placed on the forehead or depression if the negative polarity instead of the positive is placed on the same location. The indifferent electrode is usually placed distally on a leg. Since these effects seemed well substantiated (Lippold and Redfearn, 1964; Redfearn, Lippold & Costain, 1964; and Costain, Redfearn and Lippold, 1964), we had designed experiments to determine if we could condition changes in mood with external polarization as the unconditional stimulus (US). The present experiment was one of several planned to observe parallel changes which might accompany either electrically induced or conditioned depression of mood. Previous work in this laboratory has demonstrated that psychic states, such as catalepsy and catatonia, could be conditioned with classical conditioning methods (Gantt, 1953; Perez-Cruet and Gantt, 1959; and Perez-Cruet, 1966).

The main purpose of this study is to show the effects of cathodal polarization on the establishment of classical motor conditional reflexes (CRs) with faradic shock as US. Previously Gantt (1938) has shown that the conditional reflex method can measure cortical integrity. Therefore an interference with the establishment of motor conditioning by a constant polarizing current could be interpreted as a possible disruption of cortical functions.

On reviewing our literature we have been unable to find other accounts of the establishment of motor CRs in states induced by cathodal polarization of the brain such as used here, but there are other studies in humans on conditional reflex formation in depressive states (Ivanov-Smolensky, 1925; Alexander, 1961; Astrup, 1965; and Ban, 1964).

Methods and Materials

Seventeen dogs were studied. Seven experimental dogs (ESs) were constantly polarized during the establishment of classical motor CRs. Ten animals were used as controls and this group was never polarized.

In the ESs, stainless-steel wire electrodes were implanted subcutaneously and infra-orbitally as shown in Figure 1. The infra-orbital placement corresponds roughly to the location of "scalp" electrodes used by Lippold, et al. (1964). Four days after the implantation of the electrodes the ESs were trained in a soundproof room. After an initial period of camera training and orienting, constant polarizing current was passed through the head while 100 repetitions of tones, viz. conditional stimuli (CSs), were presented over a period of several days. Two tones were employed as CSs. The first tone of a frequency of 256 cycles per sec. (cps) at about 60 db level was presented for 6 sec. at the end of which an electric shock, viz. the US, was applied to the left foreleg. The electric shock was adjusted from 3 to 6 volts (5 to 7 ma.) depending on the magnitude of the reflex withdrawal of the shocked foreleg. The second tone of 512 cps was also presented for 6 sec. after an inter-trial interval of 2 min., tone 512 cps was never reinforced.

The control groups included 10 dogs some of which had been used in other experiments (Brookhouser, Perez-Cruet and Jude, 1964). They were subdivided into two groups. The first group consisted of 5 normal dogs who had been trained in the same soundproof room and with the same methods as the ESs. In order to differentiate the possible effects of surgery due to the implantation of infra-orbital electrodes, another control group, viz. the surgical control group of 5 dogs, was included.

Parameters of DC Stimulation

The DC stimulating current was connected to the ESs through the transistorized constant DC stimulation unit* as shown in Diagram 1. Four large six-volt dry-cell batteries in series were used to power the DC stimulator. The negative polarity (cathode) of the battery power supply was connected to the stimulation unit and the current was regulated from 100 μa to 2 ma. The cathodal current passing through each infra-orbital electrode was constantly monitored with separate milliameters. The positive polarity (anode) was connected to the dog's hindleg using a silver chloride electrode of about 1 in. sq. which was carefully placed on the skin with EKG paste and taped tightly with masking tape.

The polarizing current was constantly turned on while the animals were being conditioned. The conditioning sessions usually lasted 2 to 4 hours. The current levels were maintained not higher than 1 or 2 ma.

Evaluation of Motor Conditional Reflexes

The motor CRs were evaluated by careful observation of the animal's foreleg flexion or footlift response during the CSs. The criteria for motor conditioning were based on procedures previously used in our laboratory and which have been described in detail elsewhere (Gantt, 1964). Unconditional and conditional reflexes were observed and graded in a system in which no response was registered as 0 and the maximum response as +4. Intermediate responses were rated as +1, +2 and +3 respectively. Motor conditioning in most instances consisted of a reflex footlift or withdrawal of the shocked foreleg during the reinforced CS. In some experiments an accelerometer was attached to the left foreleg to measure movements.

Statistical analysis of the data was done using paired t tests. Comparison of motor CRs in controls and polarized groups was done also by portraying graphically the trial-by-trial changes in motor CRs in the first 100 reinforced trials.

Results

It was found that the normal control group formed strong (+4) motor CRs within the first 50 reinforced trials to T256 cps as shown in Figure 2. Initially most dogs showed generalization of the motor CRs during the unreinforced tone (T512), but differentiation, viz. discrimination, between T256 and T512 cps was established after 50 reinforced trials. In two dogs, Russ and Helen, strong (+4) motor CRs were established within the first 20 to 40 reinforced trials; in the others (Foxy, Peacy and Watt) after 40 to 60 reinforced trials.

In contrast, the ESs showed poor motor conditioning in 6 of the dogs. These results are shown in Figure 3, where motor CRs are plotted trial-by-trial along the abscissa; ordinate variations represent the strength of the motor CRs. Three dogs, Proton, Tres and Uno, had poor unstable motor CRs within the first 90 trials. Another two polarized dogs, Ace and Jupiter, had delayed motor CRs after 50 and 70 trials respectively, but the pattern of motor CRs was not the same as the normal control group. The dog, Bottles, only received 50 trials and within this period there was no evidence of strong motor conditioning. Only one dog, Samuel, had strong (+4) motor CRs within the first 15 reinforced trials. It is pertinent to mention that in Samuel the current density was much less than in the other dogs on account of its weight (17 Kg.) whereas the other ESs weighed from 9 to 13 Kgs. Differentiation between T256 and T512 was observed in two polarized dogs (Jupiter and Samuel). Jupiter showed almost no motor CR generalization throughout the first 100 reinforced trials even though it had strong, but delayed formation of motor CRs; Samuel showed good differentiation after 34 trials (see Figure 3). In general, however, motor differentiation was more significant statistically in the control group (d.f. = 654, t = 12.53, $p < 0.001$) than in the polarized group (d.f. = 439, t = 5.29, $p < 0.001$).

Figure 4 illustrates a histogram analysis of the frequency of 0 to +4 motor CRs in 5 normal control dogs and 5 ESs. All trials were matched for controls versus ESs and they were represented in terms of overall frequency distribution where the ordinate represents the number of motor CRs and the abscissa the various strengths of the motor CRs during T256 and T512 respectively. The histogram analysis shows a definite impairment of motor conditioning during T256. In the normal group there is a very high incidence of strong motor CRs, that is, a total of 278 (+4) motor CRs. In the polarized group the number of zero motor CRs is much greater than in the normal control group (polarized group: 224 zero motor CRs; normal control group: 65 zero motor CRs). Using paired t tests the above differences between motor CRs in control versus polarized groups were highly significant (d.f. = 658, t = 8.99, $p < 0.001$). The distribution of motor CRs during T512 cps was very similar for both groups, but still the polarized group showed more zero motor CRs (polarized group: 339 zero motor CRs; normal control group: 230 zero motor CRs).

The trial-by-trial plotting of motor CRs in the surgical control group is shown in Figure 5. In this group the dog, Wong, had a bilateral cervical vagotomy; Jeff, Esaki and Volta had intra-arterial blood pressure implants; and Boush, intra-cranial electrodes implanted in the hypothalamus. Note that the surgical control group showed strong (+3) motor CRs but these were slightly weaker than the normal control group. These differences in motor CRs between the control group and the surgical group were also highly significant (d.f. = 832, t = 6.87, $p < .001$). The surgical control group showed stronger and earlier establishment of motor CRs than the polarized group. A graphical portrayal of summation of individual motor CRs, trial-by-trial, of five dogs in each control group (non-polarized and surgical) and five dogs in the polarized group. The maximum value for the group in the ordinate is only +20 since the maximum motor CR that could be attained in each animal was set at +4. The ordinate rppresents the strength of the motor CRs; the abscissa the number of trials from the beginning of conditioning training. In the normal control group (top tracings, 1A & 1B) values of motor CRs

of +20 are observed after 45 reinforced trials. In the surgical control group (middle tracings, 2A & 2B) few values reached the maximum of +20 but values greater than +10 are observed after 35 reinforced trials. The pattern of the motor CRs in part 2B (T512) is similar to that of the normal control group. The polarized group (lower tracings, 3A & 3B) showed weaker motor CRs than either the normal or surgical control groups. The control group showed that differentiation to T512 improves later in training. The polarized group, in general, showed initially less generalization to T512 due to poor motor conditioning.

The motor unconditional reflexes (URs) were also slightly reduced during cathodal polarization as shown in Table 1. The average values for the strength of the URs in the normal control group varied from 6.4 (+3) to 84.6 (+4) and in the polarized group from 23.1 (+3) to 61.3 (+4). These differences in motor URs were statistically significant (d.f. = 993, $t = 5.32$, $p < 0.001$).

Testing the strength of the motor CRs without polarization was done after the first 100 reinforced trials under polarization in 4 dogs. Disruption of almost perfect motor differentiation was observed in only one dog, Jupiter (motor CRs to T512 under polarization were 3%; without polarization after the first 100 reinforced trials, motor CRs to T512 rose to 9%), but in 3 other dogs no clear-cut effects were observed. The strength of motor CRs to T256 after 100 reinforced trials during polarization was not impaired when the polarizing current was turned off suggesting that once the motor CRs to T256 were fully established the process was not significantly affected by the polarizing current.

Observations of the general behavior inside the soundproof room in the polarized group showed that these animals usually stood quietly during the cathodal polarization at constant current levels of 1 to 2 ma. In several experiments the ESs lowered their heads as if "depressed". This "depression-like" behavior could be inhibited by a person entering the room or by removing the dog from the soundproof room.

Discussion

The results indicate that a constant cathodal current of indefinite pathway through the brain tissue delays the establishment of classical motor conditioning. Since it has been shown that motor conditioning is dependent on the integrity of cortical functions (Gantt, 1938), one can infer from this data that central nervous system functions were temporarily affected.

It is possible that cathodal polarization acts like an external inhibiting agent. External inhibition could have acted by dampening the perception of the excitatory process during the reinforced CS. The fact that the motor UR's were slightly weaker in the polarized group than in the control groups is suggestive of external inhibition. Another possibility is that there is a change in threshold during the cathodal "depressed" state.

The observation on the development of a "depression-like" state suggests also that probably there were a number of neurophysiological changes occuring simultaneously with the subtle behavioral changes induced by the passage of a cathodal polarizing current. For example, Blagodatova's (1960) investigations

on the effects of constant current on the spontaneous EEG of the rabbit have shown that cathodal polarization on the cortex produces desynchronization of the EEG. Also Medianik and Oleinik (1957) have shown that cathodal polarization of the cerebral hemispheres reduces the excitability of salivary centers. Furthermore, the studies of Lippold, et al. (1961) have shown that polarity of evoked potentials from the sensory cortex is changed negatively or positively depending on whether the cortex is polarized positively or negatively.

It might be postulated also that the effects of cathodal polarization on motor CRs could also be mediated by an interference with the normal distribution of DC potentials as described by Burr (1961) and Becker, Bechman and Friedman (1962). These investigators have found measurable DC potentials which are generated by living organisms and which seem to control a number of biological functions such as the rate of embryonic growth and wound healing. According to Becker (1962, p. 1175) "DC potentials are particularly important in the states of consciousness or level of irritability in the human being"; therefore, if these normal DC potential gradients are altered by an imposition of "external" cathodal potentials, the state of consciousness associated with them could be affected. Hence, one could also attribute the effects of cathodal polarization on motor conditioning as due to a disruption of nervous processes at all levels including cortical and peripheral nerve endings since Becker, et al., have suggested that the DC potential gradients are also influenced by peripheral nervous processes.

Cathodal polarization of the cortex seems to impair the formation of temporal cortical connections whereas anodal polarization is known to enhance temporal connections (Rusinov, 1965, 1965) and also to enhance the retention of stimulus patterns (Morrel, 1961). However, it should be noted that experiments with anodal polarization are usually performed with implanted intracranial electrodes whereas in our study the polarizing current is spreading to the central nervous system through optical tracts and soft tissues. From our results, it appears that the effects of cathodal polarization are the suppression of motor CRs. Whether this interference with the formation of classical motor CRs is related to other processes such as "amnesia" produced by electroconvulsive therapy (Duncan, 1948; Chorover and Schiller, 1965); interaction with a "consolidation" process at the neuronal level (Hebb, 1949); or by spreading depression and inhibition of cortical processes (Bures and Buresova, 1963) remains to be shown.

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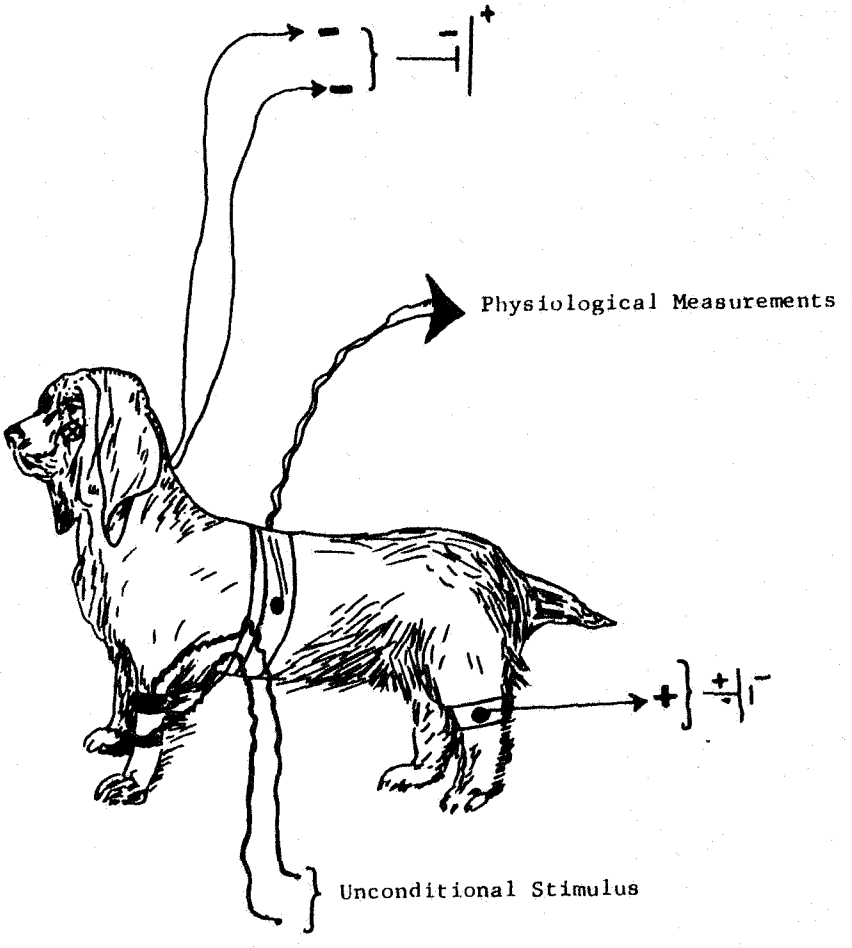

Figure 1: Illustrates placement of implanted wire electrodes in the infra-orbital region ⊗ (see curved arrow). The teflon coated stainless-steel wires were passed subcutaneously to the neck for chronic use. The indifferent electrode was placed on the hindleg. Physiological parameters (EKG and respiration) were recorded from chest electrodes. The shock to the left foreleg (US) was given through two electrodes about 1 to 1½ inches apart.

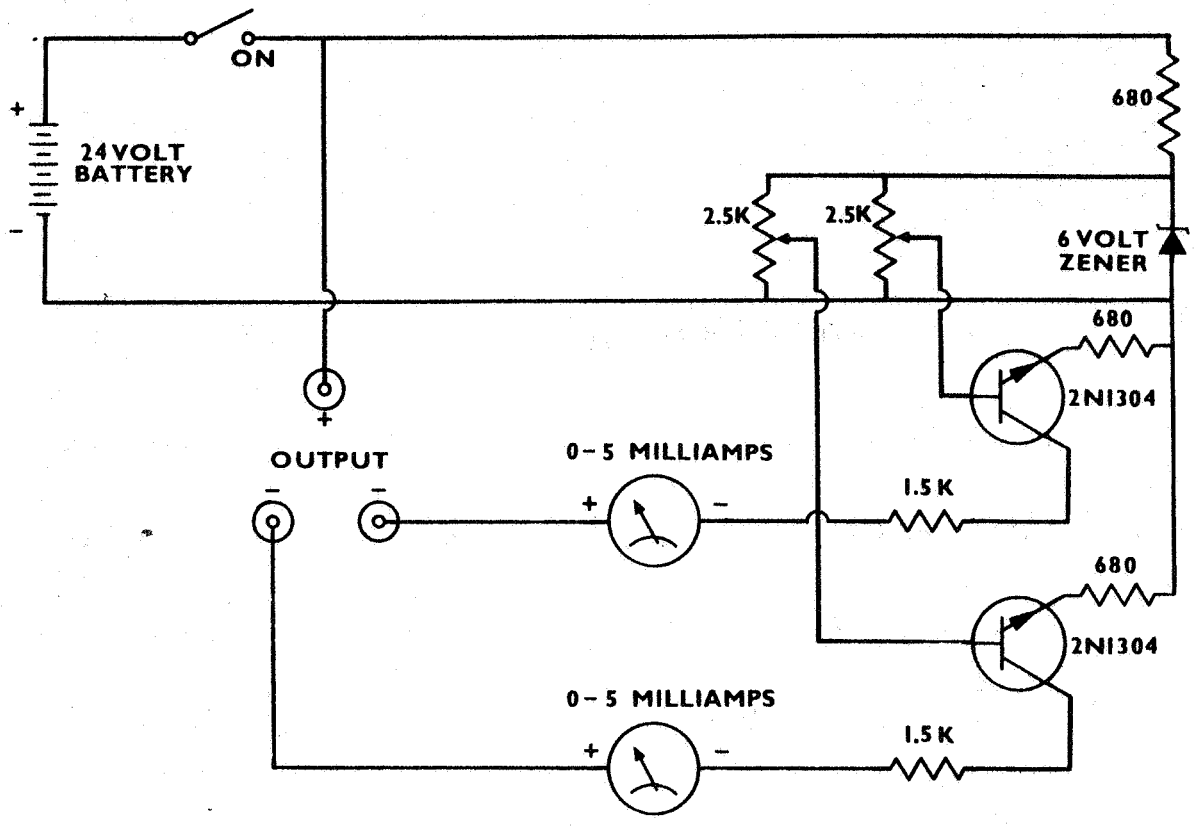

Diagram 1: Constant DC transistorized stimulation unit.

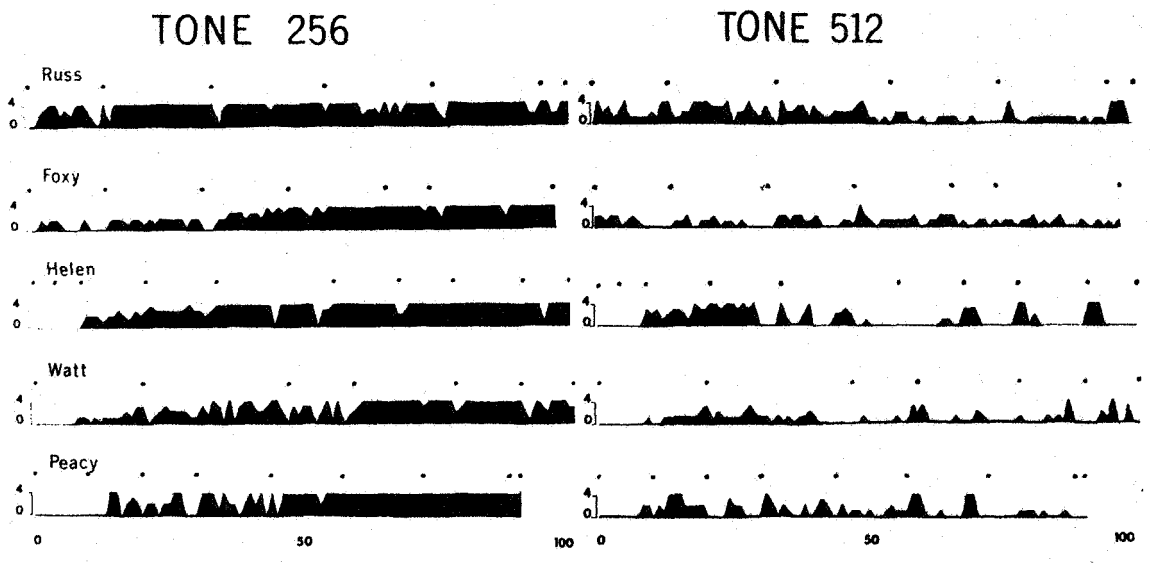

Figure 2: Changes in motor CRs in 5 normal control dogs. Abscissa indicates order of trials from 1 to 100. Ordinate indicates strength of the motor CR. Left side of the figure represents motor CRs to the reinforced tone (T256). Right side represents motor CRs during the non-reinforced tone (T512). All normal dogs showed strong (+4) motor conditioning. Dots on top of tracing indicate the number of trials per experimental session.

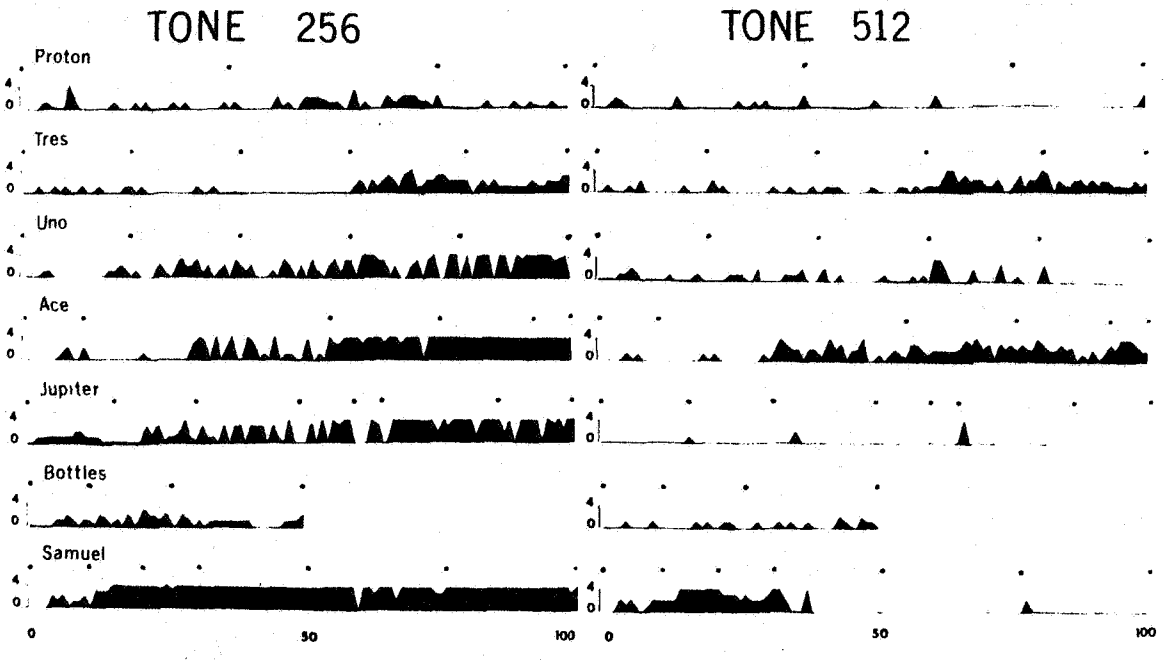

Figure 3: Changes in motor CRs in the polarized dogs (1 to 2 ma. negative DC). Abscissa and ordinate as in Figure 2. See text for explanation.

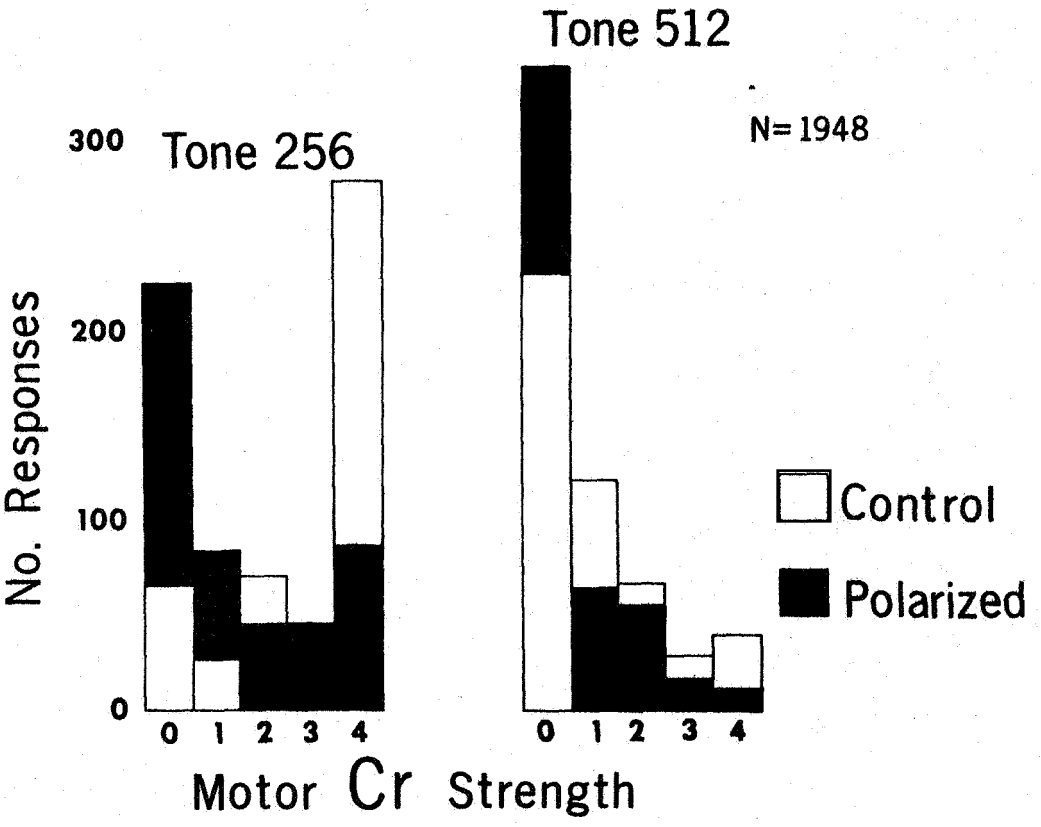

Figure 4: Histogram of motor CRs in the normal control and polarized groups during tones 256 and 512 cps. Abscissa indicates the strength of motor CRs and ordinate number of responses in a group of 5 dogs in each instance.

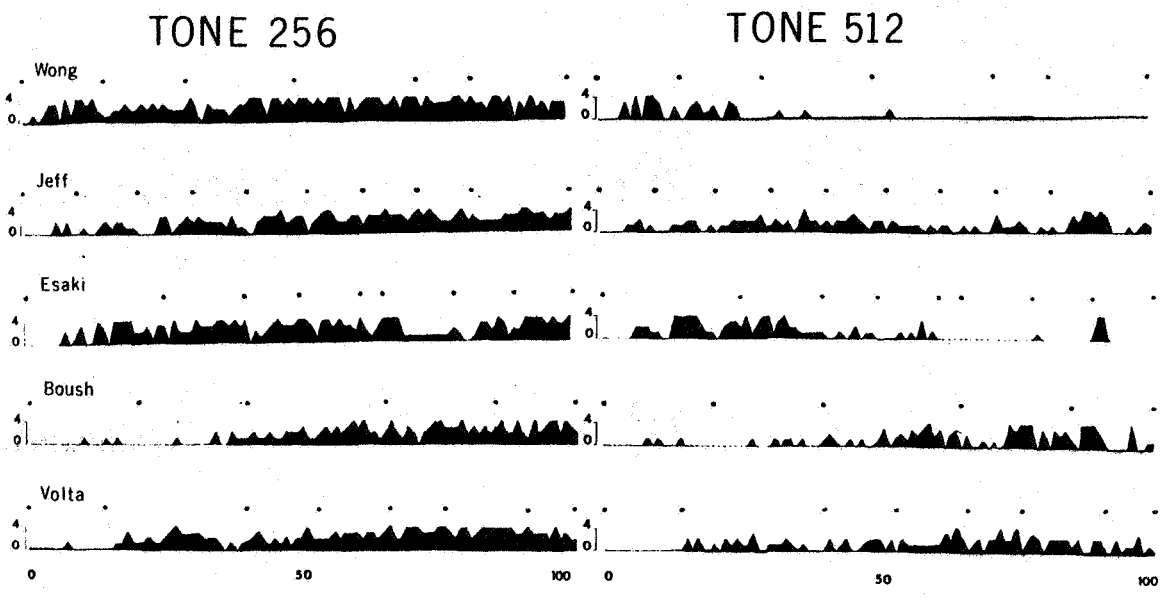

Figure 5: Changes in motor CRs in the surgical non-polarized control group. Abscissa and ordinate as in Figure 2. See text for explanation.

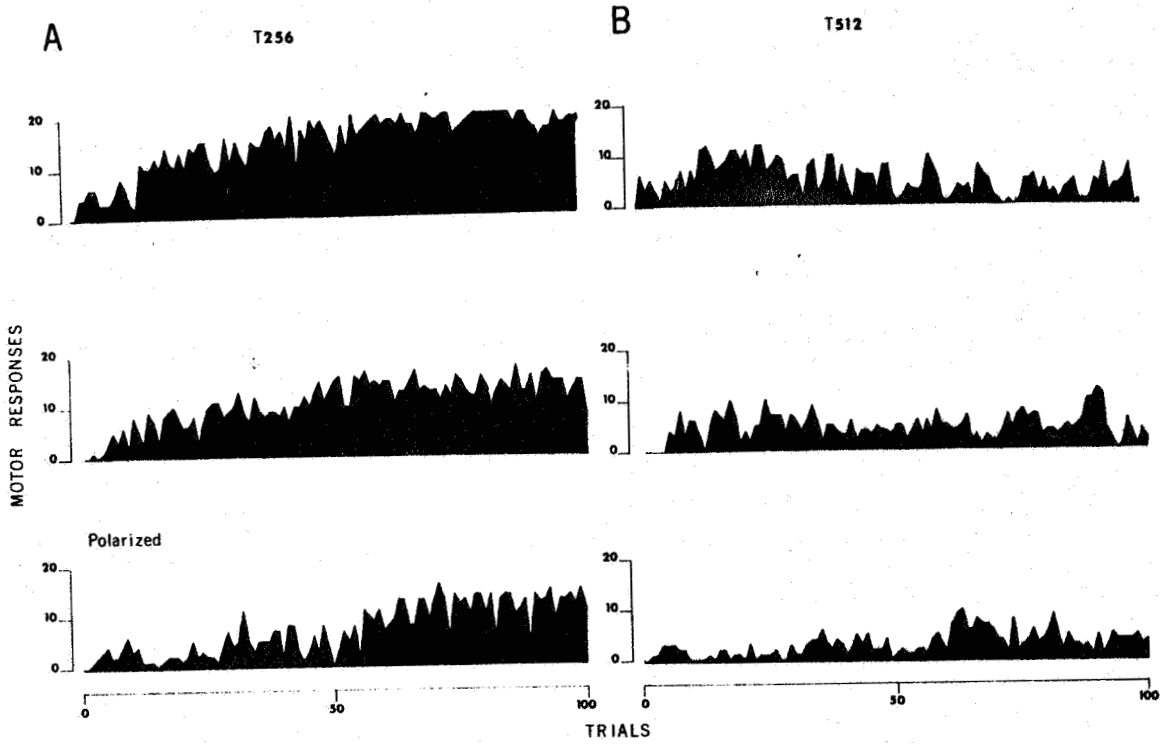

Figure 6: Changes in motor CRs in normal, surgical and polarized groups. Sum of motor CRs in five dogs were included in each tracing. See text for explanation.

TABLE I

	Dog's Name	STRENGTH OF MOTOR UNCONDITIONAL REFLEX 0	+1	+2	+3	+4
A. Control Group	1. Watt	0	1	0	2	97
	2. Foxy	1	1	2	3	92
	3. Peacy	1	1	3	0	84
	4. Russ	0	0	0	1	99
	5. Helen	3	2	18	26	51
	Total	5	5	23	32	423
	Aver.	1	1	4.6	6.4	84.6
B. Polarized Group	1. Jupiter	0	1	2	4	93
	2. Tres	0	0	2	22	75
	3. Bottles	0	3	23	21	2
	4. Proton	0	3	5	47	43
	5. Ace	0	0	4	49	47
	6. Samuel	1	0	5	8	84
	7. Uno	0	0	4	11	85
	Total	1	7	45	162	429
	Aver.	0.14	1.0	6.4	23.1	61.3

CONCLUSIONS AND SUMMARY

Psychocardiovascular reactions under terrestrial laboratory conditions can be brought under stimulus control. Methods and techniques for obtaining quantitative data were discussed in detail. Among the most promising developments, in the technical aspects, were the analysis of heart rate data in beat-to-beat for measuring short duration psychocardiovascular responses and a heart-rate cumulative method for measuring long term heart-rate changes. Blood pressure techniques for chronic measurement of absolute blood pressure were also discussed. Several cardiac and hemodynamic functions including aortic blood flow (electromagnetic), intraventricular pressures, inferior vena cava flow and renal blood flow were studied. Several patterns and types of psychocardiovascular responses were found.

Several possible models for the study of certain psychocardiovascular reactions were presented. For example, the reaction to novel stimulation (among which weightlessness or the exposure to the "psychophysiological sensation" of weightlessness could be included) and the mechanisms mediating it were presented. The novel-stimulation response was found to be mediated by the autonomic nervous system and that it represented a form of primitive adaptive response in the form of cardiac inhibition, but later this response can be modified by repeated exposure to the new situations. Similarly, painful stimulation, eliciting psychocardiovascular responses can be conditioned and it produced transient, but measurable conditional changes in cardiovascular functions with latencies of the response varying from 1 to 5 cardiac cycles. The response patterns to painful and to electrical stimulation of the brain were strinkingly similar in primates and dogs.

The physiological mechanisms mediating psychocardiovascular responses were explored by surgical removal of peripheral autonomic nerves (vagus, sympathetic) and by pharmacological blockade of parasympathetic and adrenergic mechanisms. It was found that psychocardiovascular reactions depended on the integrity of these pathways. For example it was found that the bradycardia observed during novel auditory stimulation could still be present in dogs in which the cervical vago-sympathetic trunk have been cut bilaterally. This fact indicates that the inhibitory effects of novel stimulation can be mediated by the sympathetic nervous system. Other contributing factors such as respiration and musculoskeletal inhibition played also a role in the mediation of this bradycardia response, but this is not the only mechanism because bradycardia was observed in dogs paralyzed with succinylcholine and ventilated artificially The inability to obtain psychocardiovascular responses after "denervation" shows that these responses are mediated maily by the autonomic nervous system.

The physiological effects of social interactions between a person and a primate were also studied. They were among the most striking psychocardiovascular responses mediated by the sympathetic nervous system. These findings suggest that handling of the primates by future scientist astronauts in the future orbital and space biological laboratories could be used as another simple test of the integrity of psychocardiovascular responses in animals under conditions of prolonged weightlessness.

The central mechanisms mediating psychocardiovascular reactions were explored in experiments where the hypothalamus and other intracraneal areas were electrically stimulated. Self-stimulation from these areas was obtained

in dogs and monkeys. Self-stimulation was accompanied by pronounced changes in heart rate and blood pressure. These cardiovascular changes during intracraneal stimulation can be useful in elucidating the effects of weightlessness on circulation and behavior. The response patterns produced by unconditional stimulation of the septal or posterior hypothalamic areas will define the effects of weightlessness on the differential effects of subcortical stimulation. For example, septal stimulation can produce slowing of the heart rate whereas hypothalamic stimulation can produce acceleration, therefore, it will be possible to determine if weightlessness have any effects on these responses.

The central nervous system control of certain cardiovascular and electrocardiographic irregularities was established by means of cardiovascular conditioning. A conditional electrocardiogram was produced by reinforcing a conditional stimulus with electrical stimulation of the hypothalamus. The possible interpretation that peripheral feedbacks may have been responsible for these conditional cardiovascular irregularities was not supported by experimental data because it was found that peripherally induced cardiac irregularities could not be conditioned.

The effects of vestibular stimulation on cardiovascular functions were found to be inconsistent. It is possible that during future orbital flights untreated primates could develop vertigo and undesirable vestibulo-visceral reactions which could be attributed to prolong weightlessness and orbital and vehicular orientation disturbances. It was also found that restrained primates tolerated well vestibular stimulation.

Great attention was given to the study of sinus arrhythmia because it is expected that during prolonged orbital or interplanetary travel the vagal inhibitory influences on the heart will increase, therefore, enhaning or facilitating the appearance of sinus arrhythmia. In a number of studies it was found that this type of arrhythmia is not entirely dependent on vagus tone because sinus arrhythmia was observed in bilaterally vagotomized dogs. Also sinus arrhythmia occurred independent of respiration because it was observed during panting. Various patterns of sinus arrhythmia and accompanying hemodynamic changes were studied.

Techniques for collecting urine and for measuring renal blood flow were developed. These techniques can be useful in elucidating the effects of weightlessness on renal functions.

The problem of inhibition of psychocardiovascular reactions was investigated in various experiments and species. It was found that electrically induced inhibition with cathodal electrical stimulation produced a diminution of motor conditional reflexes. Also the establishment of inhibition as an activated process was observed during classical conditioning. The problem of inhibition is an important area for future investigation because it could be useful in controlling certain psychogenic tachycardias or behavioral overexcitement.

The area of classical and operant control of cardiovascular functions was explored and it was found that these techniques may prove useful in elucidating the effects of weightlessness on psychocardiovascular reactions.

The operant control of cardiovascular functions is an area of considerable importance. By means of operant conditioning undesirable psychocardiovascular reactions could be brought under stimulus control through autogenic manipulation of visceral functions. Further investigations in this area will be required to determine its possible therapeutic application.

The practical use of some of these models and techniques will depend, in the final analysis, on whether we are really corcerned about the neural control of circulation during weightlessness or on whether pathological reactions will appear in future manned space flights. Although the scientific inquiry or theorizing may not be of paramount biomedical or pathological significance the fact remains that this basic knowledge could be useful in the exploration and conquest of space.

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